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Thermal limits of leaf metabolism across biomes

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Title: Thermal limits of leaf metabolism across biomes

Running head: Thermal limits of plants

Authors: ODHRAN S. O'SULLIVAN[1,2], MARY A. HESKEL[1,3], PETER B. REICH[4,5], MARK G. TJOELKER[4], K.W. LASANTHA K. WEERASINGHE[1,6], AURORE PENILLARD[1], LINGLING ZHU[1,11], JOHN J.G. EGERTON[1], KEITH J. BLOOMFIELD[1], DANIELLE CREEK[1,4], NUR H. A. BAHAR[1,11], KEVIN L. GRIFFIN[7], VAUGHAN HURRY[8], PATRICK MEIR[1,9], MATTHEW H. TURNBULL[10], OWEN K. ATKIN[1,11]*

Affiliations:

[1]Division of Plant Sciences, Research School of Biology, Building 46, The Australian National University, Canberra, ACT 2601, Australia

[2] Department of Animal and Plant Sciences, University of Sheffield, Alfred Denny Building, Western Bank, Sheffield S10 2TN, UK

[3]The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02544, USA

[4]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia

[5]Department of Forest Resources, University of Minnesota, 1540 Cleveland Avenue North, St. Paul, MN 55108, USA

[6]Faculty of Agriculture, University of Peradeniya, Peradeniya, 20400 Sri Lanka

[7]Department of Earth and Environment Sciences, Columbia University, Palisades NY, 10964, USA

[8]Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-901 84 Umeå, Sweden

[9]School of Geosciences, University of Edinburgh, Edinburgh EH8 9XP, UK

[10]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

[11]ARC Centre of Excellence in Plant Energy Biology, Research School of Biology, Building 134, The Australian National University, Canberra, ACT 2601, Australia

*Correspondence to: Owen.Atkin@anu.edu.au, Tel: +61 2 6125 5046, Fax: +61 2 6125 5095

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Abstract

High-temperature tolerance in plants is important in a warming world, with extreme heat-waves predicted to increase in frequency and duration, potentially leading to lethal heating of leaves. Global patterns of high-temperature tolerance are documented in animals, but generally not plants, limiting our ability to assess risks associated with climate warming. To assess whether there are global patterns in high-temperature tolerance of leaf metabolism, we quantified T_{crit} (high temperature where minimal chlorophyll *a* fluorescence rises rapidly, and thus where photosystem II is disrupted) and T_{max} (temperature where leaf respiration in darkness is maximal, beyond which respiratory function rapidly declines) in upper-canopy leaves of 218 plant species spanning seven biomes. Mean site-based T_{crit} values ranged from 41.5 °C in the Alaskan arctic to 50.8 °C in lowland tropical rainforests of Peruvian Amazon. For T_{max}, the equivalent values were 51.0 and 60.6 °C in the Arctic and Amazon, respectively. T_{crit} and T_{max} followed similar biogeographic patterns, increasing linearly (~8 °C) from polar to equatorial regions. Such increases in high temperature tolerance are much less than expected based on the 20 °C span in high temperature extremes across the globe. Moreover, with only modest high-temperature tolerance despite high summer temperature extremes, species in mid-latitude (~20°-50°) regions have the narrowest thermal safety margins in upper-canopy leaves; these regions are at the greatest risk of damage due to extreme heat-wave events, especially under conditions when leaf temperatures are further elevated by a lack of transpirational cooling. Using predicted heat-wave events for 2050 and accounting for possible thermal acclimation of T_{crit} and T_{max}, we also found that these safety margins could shrink in a warmer world, as rising temperatures are likely to exceed thermal tolerance limits. Thus, increasing numbers of species in many biomes may be at risk as heat-wave events become more severe with climate change.

Introduction

Tolerance of heat-wave events can influence the performance and fitness of organisms that experience elevated temperature (T) extremes and, due to climate change, will become an increasingly important factor in the future (Battisti & Naylor, 2009; Hansen *et al.*, 2012; IPCC, 2012). High temperature tolerance (HT_{tol}) studies in animals have found that equatorial and tropical species exhibit higher heat tolerance than those in cooler, high latitude regions (Deutsch *et al.*, 2008; Sunday *et al.*, 2011; Araújo *et al.*, 2013;). Animals in hot, low latitude environments are most at risk, although the ability to move to lower T refugia ameliorates that risk (Sunday *et al.*, 2014). HT_{tol} is also of concern for plants, which are sessile and limited in the extent to which they can ameliorate risks associated with heat waves, with sun-exposed leaves particularly vulnerable (Vogel, 2009; Leigh *et al*., 2012). Depending on factors such as leaf orientation, reflectance, transpiration rate and wind speed, leaf T can exceed ambient air T (ΔT) (Fuchs, 1990; Luquet *et al.,* 2003; Vogel, 2009; Leigh *et al.,* 2012). Leaf shape may also be crucial, with ΔT likely to be greater in broad, entire (i.e. non-dissected) leaves compared to their highly dissected and/or narrow-leaf counterparts (Givnish, 1988; Nicotra *et al*., 2011). In nature, large increases in leaf ΔT ranging from +5 to +20°C have been recorded (Ansari & Loomis, 1959; Gates *et al.*, 1968; Miller, 1972; Beadle *et al.*, 1973; Smith, 1978; Tyree & Wilmot, 1990; Valladares & Pearcy, 1997; Singsaas & Sharkey, 1998; Ishida *et al.*, 1999; Trubuzi, 2005; Doughty & Goulden, 2008; Vogel, 2009; Leuzinger *et al.*, 2010), with desert biomes unsurprisingly showing the greatest elevation likely due to the arid conditions and the lesser likelihood of transpirational cooling (Gates *et al.*, 1968; Smith, 1978); see Table S1 for details. Interestingly, in places where water availability is not limiting, such as tropical rainforests, elevated leaf temperatures of >10 °C have been recorded (Trubuzi, 2005). While such events may be rare, they can be sufficiently long in duration (Ganguly *et al.*, 2009; Perkins *et al.*, 2012) to affect plant performance and potentially plant survival (Reyer *et al.*, 2013).

Heat-waves may disrupt many plant processes (Teskey *et al*., 2014), including photosynthetic (Berry & Björkman, 1980; Hüve *et al.*, 2011) and respiratory (Hüve *et al.*, 2011; O'Sullivan *et al.*, 2013) metabolism. At the whole organism level, severe heat-waves (+12 °C above ambient) have been shown experimentally to significantly reduce biomass accumulation and net photosynthesis in red oak *Quercus rubra* and loblolly pine *Pinus taeda*, particularly in combination with drought conditions (Ameye *et al.*, 2012; Bauweraerts *et al.*, 2013, 2014). At the leaf level, short-term increases in leaf *T*s - such as those experienced during heat-wave events - increase rates of respiration, whereas net photosynthetic rates decline beyond an optimal *T* to which the plant is acclimated (Dewar *et al.*, 1999; Teskey *et al*., 2014) due to increases in CO_2 release by photorespiration and/or leaf respiration in the light exceeding carboxylation rates (Atkin *et al.*, 2006). Stomatal closure at high *T* (Valladares & Pearcy, 1997; Zweifel *et al.*, 2006) will further limit CO_2 supply and rates of net photosynthetic CO_2 uptake. Acute heat-waves may also damage photosynthetic and respiratory capacity as high *T*s increase the fluidity of leakiness of cell and organelle membranes (Hazel, 1995) leading to disruption of metabolic processes. Depending on the speed/extent of acclimation of heat tolerance - underpinned by induction of heat shock proteins, accumulation of antioxidant enzymes and osmotic agents, and changes in membrane function and chemistry (Björkman *et al.*, 1980; Seemann *et al.*, 1986; Knight & Ackerly, 2003; Sung *et al.*, 2003; Larkindale *et al.*, 2005; Sharkey, 2005; Hüve *et al.*, 2006, 2011; Velikova *et al.*, 2011) - continued exposure to heat stress may cause permanent damage to leaf tissues (Hüve *et al.*, 2011; O'Sullivan *et al.*, 2013), and contribute to hydraulic failure (Schymanski *et al.*, 2013). High *T*-mediated disruption of plant metabolic processes has been linked with adverse effects on productivity, biodiversity and crop production (Reyer *et al.*, 2013). Given these observations, warm-dry habits may provide a selective pressure for plants with a higher HT_{tol}, particularly at drier sites where the potential for evaporative cooling is diminished and where the effects of heat-waves are likely to be most acute (Bauweraerts *et al*., 2014). Consistent with this prediction, regional studies have reported higher HT_{tol} of photosynthetic

metabolism at dry, warmer sites (Knight & Ackerly, 2002, 2003). Moreover, a comparison of metabolic HT_{tol} in a diverse range of marine poikilotherms showed that heat tolerance was lower in cold-acclimated organisms compared to those from warm oceans (Hochachka & Somero, 2002). With the exception of a study documenting thermotolerance in a single forb across a latitudinal gradient (Barua *et al*., 2008), and the comparison of four congeneric species pairs from desert and coastal environments (Knight & Ackerly, 2002, 2003), the characterization of the HT_{tol} of higher plants, and how it may vary with climate and geography, is largely unknown.

To characterise HT_{tol} of leaf metabolism, many studies have focussed on the T response of minimal chlorophyll *a* fluorescence (F_o) (Schreiber & Berry, 1977; Berry & Björkman, 1980; Seemann *et al.*, 1984; Knight & Ackerly, 2002; Hüve *et al.*, 2006, 2011; O'Sullivan *et al.,* 2013). Using this approach, the T response of F_o typically follows a pattern whereby levels remained stable with moderate heating, before increasing sharply at higher Ts, followed by a sharp decline. The critical T for functioning of photosystem II (T_{crit}) is determined at the point of intersection of two lines, representing the flat and steep parts of the F_o - T response curve (Knight & Ackerly, 2002; O'Sullivan *et al.*, 2013). The increased F_o at high Ts is indicative of a disruption in electron transport due to increased membrane fluidity and dissociation of membrane-bound proteins involved in photosynthesis (Schreiber & Berry, 1977) and is associated with non-catastrophic degradation in chloroplast membranes (Hüve *et al.*, 2011). Recent studies have also characterised HT_{tol} at temperatures above T_{crit} via determination of T_{max} defined as the high T at which rates of respiratory CO_2 release are maximal (T_{max}) (Hüve *et al.*, 2011, 2012; O'Sullivan *et al.*, 2013; Gauthier *et al.*, 2014; Heskel *et al.*, 2014; Weerasinghe *et al.*, 2014). At leaf Ts above T_{max}, rates of leaf respiration irreversibly decline (O'Sullivan *et al.*, 2013), reflecting loss of mitochondrial function and the rapid onset of tissue death.

Here, we provide a cross-biome analysis of how high temperature tolerance of photosynthetic and respiratory metabolism (as characterised by T_{crit} and T_{max}) of upper canopy leaves vary with latitude and

thermal environment. Our dataset focuses on *T*-responses observed above 45°C – for patterns in the *T*-response of leaf respiration below 45°C from the same dataset, see Heskel *et al*. (2016). By relating HT_{tol} values to the magnitude and frequency of extremes in air and leaf *T*, our study also provides insights into how the *thermal safety margin* of leaf metabolism varies across the Earth's surface. Here, we define thermal safety margin as the difference between measured high *T* at which damage to leaf metabolism is detected and the leaf *T* experienced during heat-wave events – analogous to similar definitions used for animal ectotherms (Deutsch *et al.*, 2008). Our study addresses the following questions: (1) is HT_{tol} greater in plants growing in warmer compared to cooler environments; (2) does the thermal safety margin of upper canopy leaves differ among plants growing in low and high *T* environments and, if so, what regions are most at risk to high *T* stress; and, (3) what are the consequences of a future warmer world with respect to thermal safety margins?

Materials and methods

Field sites and species selection

Measurements were made at 19 globally distributed, thermally contrasting sites spanning seven biomes (Fig. 1; Table 1). The selected sites have mean annual temperatures ranging from -11 to 26 °C; across the sites, mean maximum daily temperature of the warmest month ranges from 16.7 to 36.6 °C, with heat-wave temperatures (defined as the mean maximum temperature of the warmest 3-day period) ranging from 25.2 to 44.8 °C (Table 1, Table S2). Other than one high-altitude site in Peru, the thermal environment of each site was within the 95% confidence interval for their respective 1° latitudinal band (on land) as assessed using mean maximum *T* of the warmest month taken from the *WorldClim* database (Hijmans *et al*. 2005) (Fig. S1, Table S2). Consequently, the sampled sites (other than the high altitude

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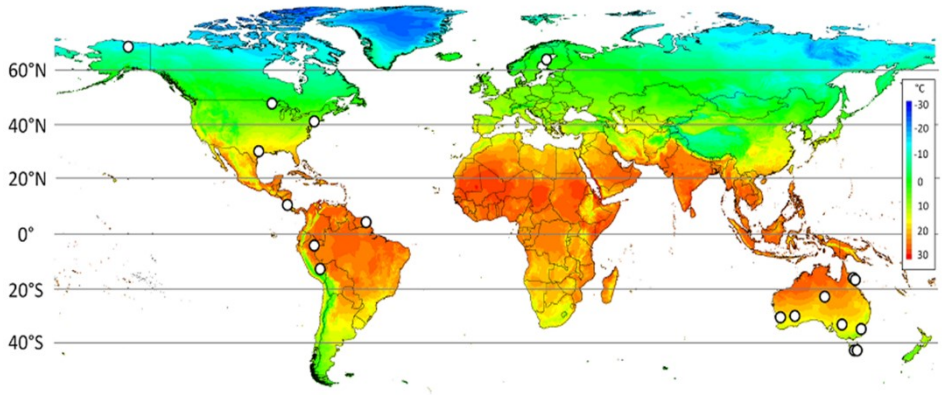

Fig. 1 Current mean annual temperature (°C) estimates from *WorldClim* data (Hijmans *et al*. 2005) with circles indicating site locations. See Table 1 for details on site locations.

Table 1 Sample sites, data collection dates, mean maximum daily temperatures of warmest months (from *WorldClim*) and heat-wave conditions of the sites (as determined from nearby meteorological stations) at which T_{crit} and T_{max} values were measured. For each site, mean values of T_{crit} and T_{max} are shown (°C). Also shown are specific leaf area (SLA; ratio of leaf area to leaf mass – m^2 kg^{-1}), and concentrations of leaf nitrogen (N, mg g^{-1}) and leaf phosphorus (P, mg g^{-1}). Note that no data are available for [N] and [P] at several sites (--). Sites are listed in order of highest to lowest absolute latitude (i.e. from poles to equator). Altitude (alt, metres above sea level), number of species (*n*) and biome classes at each site are also shown. Biome classes: BF, boreal forests; TeDF, temperate deciduous forest; TeRF, temperate rainforest; TeW, temperate woodland; TrRF_lw, lowland tropical rainforest (<1500 asl); TrRF_up, upland tropical rainforest (>1500 asl); Tu, tundra. Other abbreviations: HWRC, Hubecheck Wilderness Research Center; TAS, Tasmania; ACT, Australian Capital Territory; ANU, Australian National University; WA, Western Australia; NT, Northern Territory; QLD, Queensland. T_{crit} and T_{max} refer to the high *T* tolerance of photosynthesis and leaf respiration, as defined in the main text.

Site	Biome	Location		alt	*n*	Sampling date	Mean max daily *T* of warmest month	Mean max *T* of warmest 3-day period during 2001-2010	T_{crit}	T_{max}	*SLA*	*[N]*	*[P]*
		°N	°E	m	spp		°C	°C	°C	°C	(m^2 kg^{-1})	(mg g^{-1})	(mg g^{-1})
Toolik, Alaska	Tu	68.38	-149.36	720	20	Jun 2010	16.7	25.2	41.5	53.0	11.8	22.55	2.01
Umea, Sweden	BF	63.82	20.31	29	11	Aug 2013	20.5	29.0	46.4	54.0	10.0	16.45	1.28
HWRC, Minnesota	BF	47.96	-91.75	420	15	Jul 2013	25.6	34.1	44.9	54.9	11.1	17.44	1.38
Warra, TAS low altitude	TeRF	-43.10	146.72	88	2	Mar 2012	20.7	35.0	43.5	55.7	5.4	8.19	0.42
Warra, TAS high altitude	TeRF	-43.09	146.67	237	10	Mar 2012	20.4	34.6	43.7	58.3	7.3	14.44	0.60
Black Rock Forest, New York	TeDF	41.41	-74.01	335	10	Jun 2013	27.1	37.2	47.3	55.6	14.6	21.62	1.59
Acton, Canberra, ACT	TeW	-35.28	149.11	571	4	Mar 2012	28.2	39.9	-	55.9	15.3	--	--
Aranda, Canberra, ACT	TeW	-35.28	149.08	580	10	Oct 2011	27.9	39.9	-	51.0	5.4	--	--
Calperum, SA	TeW	-34.04	140.67	35	18	Mar 2013	32.7	44.8	49.5	60.6	3.9	13.20	0.61
Texas A&M, Texas	TeW	30.60	-96.40	103	2	Oct 2010	35.5	40.8	-	55.2	4.7	--	--
Great Western Woodlands, WA	TeW	-30.26	120.69	459	16	Apr 2013	34.3	43.9	49.7	58.2	5.7	17.77	0.66
Jurien Bay, WA	TeW	-30.22	115.04	9	11	Nov 2011	31.3	43.3	42.4	57.8	6.8	14.36	0.80
Alice Mulga, NT	TeW	-22.28	133.25	607	5	Feb 2013	36.6	43.0	50.1	60.0	3.8	12.58	0.73
Atherton, QLD	TrRF_lw	-17.12	145.63	728	16	Aug 2012	29.1	37.0	46.2	55.7	9.3	16.27	1.16
Cape Tribulation, QLD	TrRF_lw	-16.28	145.48	90	12	Sep 2010	31.9	37.3	-	59.4	9.2	2.20	0.10
Andes, Peru	TrRF_up	-13.19	-71.59	3000	16	Sep 2011	22.4	20.8	42.9	53.8	6.6	17.77	1.78
Costa Rica	TrRF_lw	10.38	-84.62	479	6	Jul 2011	30.7	33.4	-	58.5	19.8	--	--
Paracou, French Guiana	TrRF_lw	5.27	-52.92	21	37	Oct 2010	31.3	34.5	50.4	58.5	10.1	15.44	0.62
Iquitos, Peru	TrRF_lw	-3.95	-73.43	114	19	Sep 2011	32.0	37.5	50.8	59.6	7.8	17.18	0.75

site) were not outliers in relation to thermal environment with latitude; the high altitude site in Peru was therefore used in analyses related to growth T but not to latitude.

For each of the 19 sites, measurements were conducted over a 1- to 3-week period during one of the warmest months of the year (Table 1). A single leaf from three to four replicate individuals of a representative species were selected for measurement at each site, with the exception of the two Peruvian sites where only one replicate per species was selected due to time and logistical limitations (corresponding to 16% of the species in the dataset). Upper canopy, sun-lit leaves were sampled and stored in cool, moist dark conditions until measurement of T-responses, typically within six hours of sampling. In total, we developed a global HT_{tol} data set comprising 798 individual measurements of 218 plant species representing a range of plant functional types including evergreen and deciduous tree species, evergreen shrubs and forb species. The vast majority of the selected species were woody (203 sp.), with 15 non-woody species being sampled at three sites (Toolik, Alaska and the two sites in Western Australia). Finally, to assess whether T_{crit} and T_{max} seasonally acclimate, additional measurements were made on a three species at the Greater Western Woodland site in Western Australia (*Olearia mulleri, Ptilotus holosericeus* and *Sclerolaena diacantha*), and at the Atherton tropical wet forest site in Far North Queensland (*Alstonia meulleriana, Cryptocarya mackinnoniana* and *Gillbeea adenopetala*); these measurements were made at time points approximately 5-6 months offset from that of the original campaigns. Thus, for the above listed species, T_{crit} and T_{max} values were available during the warm and cool seasons at each site.

Determination of high temperature tolerance (HT_{tol})

We measured two aspects of HT_{tol} previously linked to changes in cell membranes and plant metabolic performance: heat-induced changes to minimal chlorophyll *a* fluorescence (F_o) - T_{crit} (Schreiber & Berry, 1977); and the upper thermal limit of leaf respiratory CO_2 release in darkness - T_{max} (Hüve *et al.*, 2011;

O'Sullivan *et al.*, 2013). Whole leaves were placed in a *T*-controlled, well-mixed cuvette; the cuvettes were *T*-controlled via a thermostatically-controlled circulating water bath as in O'Sullivan *et al.* (2013) and Heskel *et al.* (2014), or via a Peltier system (3010-GWK1 Gas-Exchange Chamber, Walz, Heinz Walz GmbH, Effeltrich, Germany). After a 30-min dark adaption period, the cooled cuvette chamber was heated continuously at a rate of 1°C min^{-1}, until T_{max} was reached (generally leaf *T* between 55-70°C). Leaf *T* was measured with a small-gauge wire copper constantan thermocouple pressed against the lower surface of the leaf, and which was attached to a LI-6400 external thermocouple adaptor (LI6400-13, Li-Cor Inc., Lincoln, NE, USA) that enabled leaf *T*s to be recorded by a LI-6400XT portable gas exchange system (Li-Cor Inc.).

During each *T*-response experiment, we measured F_o in the presence of a low intensity far-red light pulse (necessary to maintain PSII in the oxidized state) every 30 s using a MiniPAM portable chlorophyll fluorometer (HeinzWalz, Effeltrich, Germany) fitted to the glass surface of the leaf chamber. T_{crit} was determined at the point of intersection of two regression lines, representing the flat and steep parts of the F_o - *T* response curve (Knight & Ackerly, 2002; O'Sullivan *et al.*, 2013). The linear parts of each curve were determined by calculating the instantaneous slope of the relationship between F_o (normalised so that maximum value equals 100) and *T* across a 3°C range centred on the measurement temperature. The regression lines for the flat and steep parts of the curve were calculated using points where the slope values were <1 or >3.5 respectively. These values were chosen arbitrarily but used consistently to ensure comparable T_{crit} values. Respiratory CO_2 release was also recorded at 30s intervals using a LI-6400XT portable gas exchange system [fitted with an empty and closed 3 x 2 cm cuvette (Li-Cor 6400-02B)] that was plumbed into the air-stream exiting the leaf chamber. T_{max} was identified as the high leaf *T* where rates of respiratory CO_2 release were maximal (O'Sullivan *et al.*, 2013). Post-measurement, each leaf was oven dried at ~60°C for a minimum of two days and weighed. To enable calculation of specific leaf area (SLA, ratio of leaf area to leaf dry mass with units of m^2 kg^{-1}), we

measured the leaf area [using either a LI-3100C leaf area meter (Li-Cor Inc.) or *Image J* (www://imagej.nih.gov/ij/) analyses of a scanned leaf image] and dry mass of an adjacent leaf. Where stated, total nitrogen (N) and phosphorus (P) concentrations were also determined using Kjeldahl digests (Allen et al., 1974) that were analysed using a LaChat Quikchem 8500 Series 2 Flow Injection Analysis System (Lachat Instruments, Milwaukee, WI, USA).

Meteorological data

Local meteorological station data (using the nearest meteorological station at a comparable altitude; Table S2) from 2001-2010 were used to examine trends associated with actual thermal conditions and recent heat-waves experienced at each site, rather than exclusively assessing trends based on changes with latitude as is often done in non-plant organisms (Deutsch *et al.*, 2008; Sunday *et al.*, 2011; Araújo *et al.*, 2013). Restricting meteorological station data to 2001-2010 enabled standardization of data across stations (which vary in the number of years available), while also providing a common review period for all sites. In the case of two sites (Toolik, Alaska and Andes, Peru) meteorological station data from nearby field stations were used (Trubuzi, 2005; K. Halladay and Y. Malhi personal communication). For other sites, meteorological station data were collected from publically available records accessed from national government websites (Miller, 1972; Tyree & Wilmot, 1990; Ishida *et al.*, 1999). In most cases, data were collected from a station within 100 km with an elevation difference of <100 m, with the exception of the sites in Costa Rica and Atherton, QLD (Table S2).

The use of local meteorological data allowed for the assessment of recent heat-wave events to calculate the thermal safety margin of HT_{tol} for T_{crit} and T_{max} referenced against air T. The thermal safety margin was defined as the difference between the HT_{tol} of each species at each site and the observed heat-wave extremes, with latter being the mean maximum air T of the warmest three-day period at each site

223 from 2001-2010 (Table 1). (*Note*: other ways of representing maximum *T*s alongside details of met
224 stations for each site are shown in Table S2).

225 Current modelled climate data for each were collected from the *WorldClim* database (Hijmans *et*
226 *al.*, 2005) at 2.5 arc-minutes resolution. Future modelled climate data were also collected from the
227 *WorldClim* website using the Hadley Centre model (HadGEM2-ES). Future heat-wave events for 2050
228 were estimated assuming that the warmest 3-day heat-wave event for 2001-2010 at each site would
229 increase in parallel with predicted increases in the mean maximum *T* of the warmest month by 2050 as
230 predicted in the high-emission representative concentration pathway (RCP) 8.5 IPCC scenario, the
231 scenario which most closely matches current emission estimates (Friedlingstein *et al.*, 2014). The RCPs
232 are four emission scenarios that range from early mitigation (RCP2.6) to very high baseline emissions
233 (RCP8.5) that provide high resolution spatial data on future climate conditions (corresponding to
234 radiative forcing values from 2.6 to 8.5 W/m^2) (Vuuren *et al*., 2011).

235

236 *Data analysis*

237 Bivariate regression was used to explore relationships between HT_{tol} values (T_{max} and T_{crit}) and latitude,
238 and with various measures of the thermal environment. For the latter, the correlations with the highest r^2
239 were against mean maximum *T* of the warmest month (MTWM). To test if relationships between HT_{tol}
240 and latitude/MTWM differed between sites in the northern and southern hemispheres, we analyzed the
241 data sets using analysis of covariance (ANCOVA), with latitude or MTWM as the covariant (Fig. S2).
242 Similarly, ANCOVA was used to test whether relationships between HT_{tol} and latitude and MTWM
243 differed between evergreen and deciduous species (Fig. S3). These analyses were conducted using SPSS
244 Statistics V22 (IBM Corp Armonk, NY, USA).

245 Given the observed scatter in T_{max} and T_{crit} value at any given latitude, and the clear role that
246 altitude plays (e.g. Andes site in Peru), it may be that much of the variation in observed values could be

247 accounted for by including altitude in a model, or more simply site temperature (which is influenced by
248 latitude and altitude). Similarly, the variation in observed values might reflect the impact of
249 environmental factors other than mean maximum T of the warmest month, such as drought, on T_{max} and
250 T_{crit}. With these issues in mind, we used backward-stepwise regression to select best-fitting equations
251 from a starting set of input climate variables, using site-mean values of T_{max} and T_{crit}. To explore
252 relationships between HT_{tol} and associated leaf structural and chemical composition traits, we conducted
253 additional backward-stepwise regressions to select best-fitting equations from a starting set of both input
254 climate variables and the following leaf traits: specific leaf area (SLA), leaf [N] and [P]; for theses
255 analyses, we used both site-mean and species-mean values of the above traits. In all of the above cases,
256 parameters were chosen that exhibited variance inflation factors (VIF) <2.0 (i.e. minimal co-linearity);
257 *F*-to-remove criteria were used to identify best-fitting parameters. Multiple regression was then used to
258 estimate predictive equations for the chosen variables. The *PRESS* statistic (predicted residual error sum
259 of squares) was used to provide a measure of how well each model predicted T_{max} and T_{crit} values; the
260 *PRESS* statistic provides a measure of how well each regression model predicts the observations, with
261 smaller *PRESS* indicating better predictive capability. Relative contributions of climate variables to each
262 regression were gauged from their standardized partial regression coefficients. Stepwise and associated
263 multiple linear regressions were conducted using Sigmaplot Statistics v12 (Systat Software Inc., San
264 Jose, CA, USA).

265 In the absence of extensive data documenting acclimation of T_{crit} and T_{max} in diverse assemblages
266 of species (but see Results section for seasonal acclimation data at two sites), we calculated T_{crit} and T_{max}
267 in a future, warmer world (taking into account acclimation) assuming that: (1) the existing biogeographic
268 patterns in T_{crit} and T_{max} mirror those of phenotypic adjustments in response to differences in growth T
269 experienced at each site – in doing so, we adopted an approach similar to that taken for modelling
270 acclimation responses of leaf respiration using a global respiration data set (Atkin *et al.,* 2015; Slot &

271 Kitjima, 2015; Vanderwel *et al.*, 2015); (2) that all species would acclimate to future increases in T to
272 the same extent; and (3), that there are no limits to the degree of high T acclimation.

273 Based on these assumptions, we calculated future T_{crit} values using the formula:

274 $$\text{acc}T_{crit} = T_{crit} + (\Delta T \cdot m)$$

275 whereby $accT_{crit}$ is the acclimation value for T_{crit}, ΔT is the difference between the mean maximum T of
276 the warmest month under current and future RCP8.5 conditions for each site, and m is the slope of the
277 relationship between T_{crit} and mean maximum T of the warmest month. The same formula was used to
278 account for acclimation in T_{max} using the slope of the relationship between T_{max} and mean maximum T
279 of the warmest month. Using this approach, we note that increases in HT_{tol} may not be linear with respect
280 to future increases in growth T; rather HT_{tol} might be expected to decrease disproportionally as growth T
281 increases above an optimum (Barua *et al.,* 2004, 2008; Wang *et al.,* 2008). While this may mean that our
282 approach over-estimates acclimation of HT_{tol} to rising growth Ts, it also had the benefit of providing a
283 conservative estimate of deleterious effects of future increases in growth T on the thermal safety margin
284 of HT_{tol}.
285
286

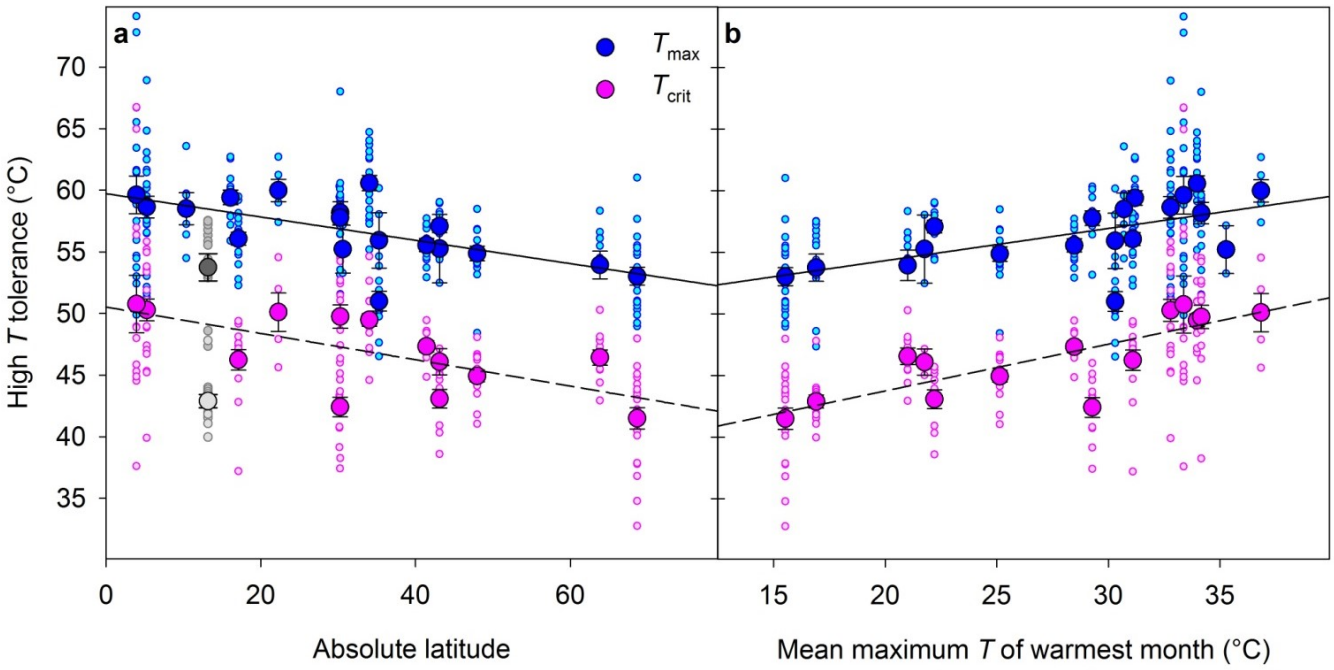

Fig. 2 Global patterns in high temperature (T) tolerance in plants for 171 (T_{crit}) and 218 (T_{max}) different species. Linear regressions show (**a**) absolute latitude and (**b**) mean daily maximum T of the warmest month (MTWM) are significantly correlated ($p < 0.01$) with of T_{crit} (dashed lines) and T_{max} (solid lines). Equations for linear regressions through site mean data: Fig. 2a, T_{crit} = 50.498 - 0.1061*latitude, T_{max} = 59.973 - 0.0984*latitude; Fig. 2b, T_{crit} = 36.138 – 0.3805*MTWM, T_{max} = 49.1545 – 0.2643*MTWM (see Table 2 for further details). Larger points indicate site means ± SE with species mean data indicated in smaller points. In (a), values for a tropical high altitude site in Peru excluded from latitude regression analysis are indicated in light (T_{crit}) and dark (T_{max}) grey symbols. Details on each site MTWM are shown in Table 1 & Figure S6.

Results

Current biogeographical patterns in HT_{tol} and thermal safety margins

HT_{tol}, as measured by T_{crit} and T_{max}, was greater at lower latitudes (Fig. 2a) and at warmer sites (mean maximum T of the warmest month of the year) (Fig. 2b), noting that growth T co-varies with latitude. Mean site-based T_{crit} was 10.5 ± 0.6 °C lower than mean T_{max}, but both followed similar biogeographic

295

Table 2 Equations of linear relationships shown in Fig. 2

Linear relationship (y = mx + c)					
y	x	m	c	r^2	p
T_{crit}	|latitude|	-0.1061	50.4975	0.4356	0.0141
T_{max}	|latitude|	-0.0984	59.9727	0.4380	0.0028
T_{crit}	Mean max T (warmest month)	0.3805	36.1375	0.6603	0.0004
T_{max}	Mean max T (warmest month)	0.2643	49.1545	0.3807	0.0049

Table 3. Regression equations expressing T_{max} and T_{crit} as function of site climate and leaf traits, using site-mean values of leaf traits. n = number of sites. To select the best fitting equation from a group of input independent variables, data were explored using *Backwards-Stepwise Regression* – this revealed that chosen parameters exhibited *variance inflation factors* (VIF) less than 2.0 (i.e. minimal multi-collinearity); it also identified best-fit parameters (using F-to-remove criterion). Thereafter, multiple regression analyses were conducted to estimate predictive equations for the chosen variables. All selected variables were significant (P<0.001). The *PRESS* statistic (predicted residual error sum of squares) provides a measure of how well each regression model predicts the observations, with smaller *PRESS* indicating better predictive capability. Relative contributions of location and climate variables to each regression can be gauged from their standardized partial regression coefficients (β_1-β_2, depending on model equation). Abbreviations: aridity index (AI) calculated as the ratio of mean annual precipitation to mean annual potential evapotranspiration (UNEP, 1997, Zomer *et al.*, 2008); mean maximum temperature of the warmest month (MTWM); specific leaf area (SLA; ratio of leaf area to leaf mass – $m^2\ kg^{-1}$); leaf nitrogen (N, mg g^{-1}); leaf phosphorus (P, mg g^{-1}). MTWM at each site were obtained using site information and the *WorldClim* data base (Hijmans *et al.*, 2005)

			Multiple linear regression parameters				
Dependent variable	Input: independent variables *(Backwards-Stepwise Regression)*	Output: selected equations *(Multiple Linear Regression)*	n	r^2	*PRESS* statistic	Standardized partial regression coefficients β_1	β_2
T_{crit}	Latitude, altitude, aridity index (AI), SLA, N, P	T_{crit} = 50.912 - (0.100 * Latitude) - (0.00215 * Altitude)	14	0.494	99.1	-0.616	-0.504
	MTWM, aridity index (AI), SLA, N, P	T_{crit} = 36.137 + (0.38 * MTWM)	14	0.660	56.9	0.813	
T_{max}	Latitude, altitude, aridity index (AI), SLA, N, P	T_{max} = 60.494 - (0.0960 * Latitude) - (0.00182 * Altitude)	19	0.521	79.4	-0.648	-0.447
	MTWM, aridity index (AI), SLA, N, P	T_{max} = 49.155 + (0.264 * MTWM)	19	0.381	93.8	0.617	

296

297 patterns. These significant and parallel linear relationships with geographic and climatic origins (Table
298 2) were maintained when considering the northern and southern hemisphere sites separately (Fig. S2).
299 Similarly, an analysis of covariance (with latitude or mean maximum T of the warmest month of the year
300 as co-variants) revealed there was no main effect of whether species were categorized as evergreen or
301 deciduous, indicating that the above HT_{tol} patterns are unlikely to be dependent on leaf growth habit (Fig.
302 S3). Although we focus on the high T of the warmest months, the positive relationship of HT_{tol} with air

303

Table 4 Percentage of species measured which exceed the T_{crit} thermal safety margin (i.e. $T_{leaf} > T_{crit}$) during heat-waves under current and future (RCP8.5 in 2050) climate scenarios. For both 'Current' and 'Future (RCP8.5 in 2050)' scenarios, the percentage of species exceeding T_{crit} are shown for events where the elevation of leaf temperature (T_{leaf}) over that of the surrounding air temperature is calculated at +0, +5 °C and +10 °C

Site	*Biome*	*Location*		*Scenario*					
				Current			Future (RCP8.5 in 2050)		
				Elevation in T_{leaf} (°C)			*Elevation in T_{leaf} (°C)*		
				+0	+5	+10	+0	+5	+10
		°N	°E						
Toolik, Alaska	Tu	68.38	-149.36	0.0	0.0	10.0	0.0	0.0	15.0
Umea, Sweden	BF	63.82	20.31	0.0	0.0	0.0	0.0	0.0	0.0
HWRC, Minnesota	BF	47.96	-91.75	0.0	0.0	20.0	0.0	6.7	86.7
Warra, TAS – low altitude	TeRF	-43.10	146.72	0.0	0.0	50.0	0.0	0.0	50.0
Warra, TAS – high altitude	TeRF	-43.09	146.67	0.0	10.0	60.0	0.0	30.0	100.0
Black Rock Forest, New York	TeDF	41.41	-74.01	0.0	0.0	55.6	0.0	11.1	100.0
Acton, Canberra, ACT	TeW	-35.28	149.11	-	-	-	-	-	-
Aranda, Canberra, ACT	TeW	-35.28	149.08	-	-	-	-	-	-
Calperum, SA	TeW	-34.04	140.67	5.6	66.7	100.0	5.6	88.9	100.0
Texas A&M, Texas	TeW	30.60	-96.40	-	-	-	-	-	-
Great Western Woodlands, WA	TeW	-30.26	120.69	6.3	18.8	87.5	6.3	62.5	100.0
Jurien Bay, WA	TeW	-30.22	115.04	54.5	100.0	100.0	72.7	100.0	100.0
Alice Mulga, NT	TeW	-22.28	133.25	0.0	40.0	80.0	0.0	40.0	100.0
Atherton, QLD	TrRF_lw	-17.12	145.63	0.0	7.1	42.9	7.1	14.3	85.7
Cape Tribulation, QLD	TrRF_lw	-16.28	145.48	-	-	-	-	-	-
Andes, Peru	TrRF_up	-13.19	-71.59	0.0	0.0	0.0	0.0	0.0	0.0
Costa Rica	TrRF_lw	10.38	-84.62	-	-	-	-	-	-
Paracou, French Guiana	TrRF_lw	5.27	-52.92	0.0	0.0	4.8	0.0	4.8	14.3
Iquitos, Peru	TrRF_lw	-3.95	-73.43	0.0	7.7	38.5	7.7	15.4	61.5
		Mean of all sites		**4.5**	**17.5**	**42.9**	**6.8**	**27.7**	**61.6**

304 *T* is similar for a variety of metrics (e.g. mean annual *T*). Furthermore, in no case did accounting for site
305 aridity significantly improve model fits over and above fits obtained using data on location or climate,
306 when using site-mean values (Table 3). Similarly, accounting for leaf traits central to the 'leaf economic
307 spectrum' (Wright *et al*., 2004) - that being specific leaf area (SLA), and mass-based concentrations of
308 leaf nitrogen ([N]) and phosphorus ([P]) - did not improve model fits in analyses that used site-mean
309 values (Table 3). When using species-mean data, accounting for the above leaf traits also did not improve
310 model fits for T_{crit} (Table S5), suggesting that HT_{tol} of photosynthesis is not linked to leaf traits strongly
311 linked to growth potential; by contrast both mean maximum temperature of the warmest month (MTWM)
312 *and* leaf [P] were retained the preferred model for T_{max}, when using species-mean values (Table S5).

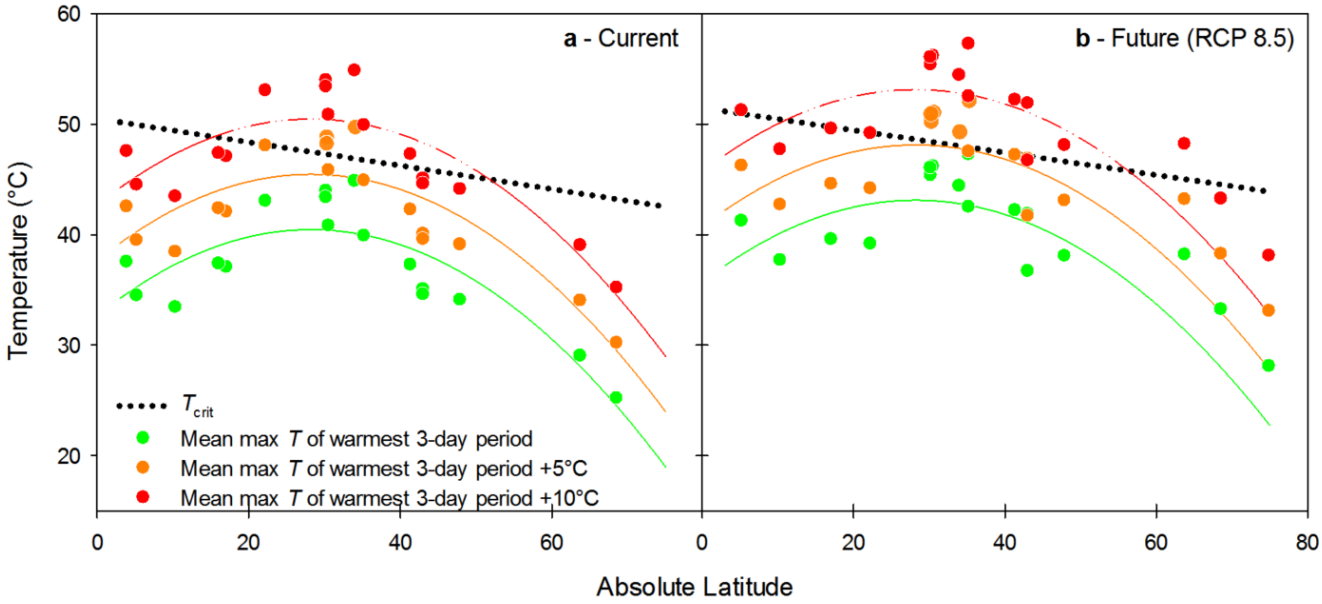

Fig. 3 Current and future heat-wave temperature (*T*) extremes and relation to global patterns in T_{crit}. Mean maximum air *T* of the warmest three-day period (**a**) recorded at each site and (**b**) predicted for 2050 under RCP 8.5, with +5 °C and +10 °C above ambient included to illustrate scenarios in which leaf *T* further exceeds air *T*. Curved lines show relationship of site heat-wave *T*s with latitude; bold dotted line (·····) shows the mean T_{crit} change with latitude derived from Fig. 2a. Broken dash-dot lines (–··–) indicate latitudes where leaf *T*s exceed T_{crit} (e.g. when predicted leaf *T* is +10 °C greater than air *T*). Excluded from the figure are data from the tropical, high-altitude site in Peru.

313 Both T_{crit} and T_{max} ranged ~8 °C from arctic to equatorial sites (Fig. 2). This 8 °C range in HT_{tol}
314 is narrow when compared to the 20 °C range in mean maximum daily *T* of the warmest month from arctic
315 to equatorial sites (Table 1), suggesting that thermal safety limits of T_{crit} and T_{max} are not constant across
316 the globe. To assess whether this might be true, we quantified geographic variation in heat-wave extremes
317 using the mean maximum air *T* of the warmest recorded three-day period of each site from 2001-2010
318 (Fig. 3a) because these represent extreme events that might result in thermal leaf damage. The current
319 station data (Fig. 3a) show that highest heat-wave *T*s do not occur in equatorial tropical forests where
320 there is high potential for loss of latent heat during evaporation (Strahler & Strahler, 1989); rather, the
321 highest heat-wave *T*s occur at the relatively dry, inland sites at mid latitudes (20-40°) (Fig. 3a). Air *T*s
322 during these periods approach mean HT_{tol} (for T_{crit}) thresholds at each site (Fig. 3a) and exceed some
323 individual species HT_{tol} thresholds (Table 3). If leaf *T*s exceed air *T*s (e.g. by +5 or +10 °C; Fig. 3a), as

can sometimes occur (Table S1), HT_{tol} (for T_{crit}) thresholds are breached for greater numbers of species (Table 4), surpassing some site means (Fig. 3a), with species in mid latitude sites (20-50°) most at risk. Greater numbers of species are at risk under heat-wave scenarios predicted under RCP8.5 (Fig. 3b). The narrowest thermal safety margins (calculated using the mean maximum heat-wave air T recorded at each site) occur in species at mid-latitude (~20° to 50°) sites (Fig. 4a; Figs S3 & S4), mirroring global heat-wave patterns (Fig. 3a). Thus, although HT_{tol} increases with increasing mean maximum T of the warmest month (Fig. 2b), such increases in HT_{tol} are insufficient to maintain geographic homeostasis of the thermal safety margin at mid-latitude sites where the severity of heat-waves is most pronounced.

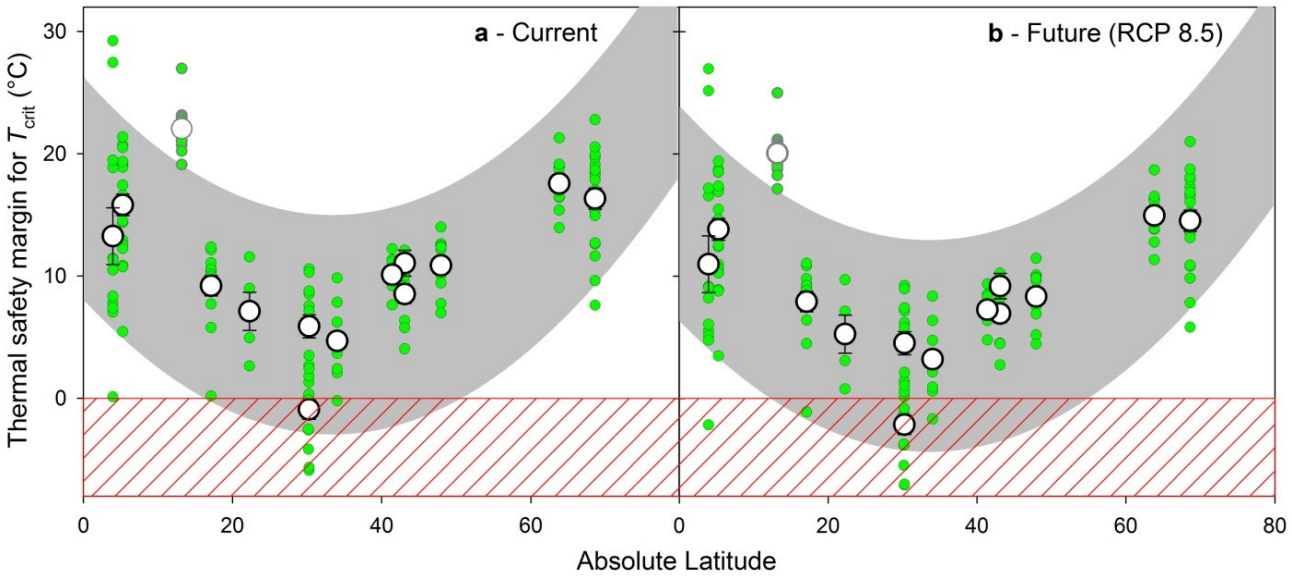

Fig. 4 Thermal safety margins (TSM) of T_{crit}, assuming leaf temperature (T) equals air T, both now and in the future. TSMs determined using: (**a**) observed values of T_{crit} (Fig. 2a) and mean maximum daily temperature (T) over warmest consecutive 3-day period from 2001-2010 (Fig. 3a); and, (**b**) predicted future values of T_{crit} (accounting for potential thermal acclimation) and estimated future mean maximum 3-day heat-wave temperatures under RCP 8.5 (Fig. 3b) for each measured species at each site. Grey shading indicates 95% CI of TSM across latitudes. Red hatched box when TSM < 0 and so corresponds to the leaf injury zone at which T_{crit} has been exceeded when no elevation in leaf T has been assumed. White/open symbols indicate site means ± SE. Green/closed circles indicate individual species mean TSM values at each site. Site-mean values for a high altitude site in the Peruvian Andes (excluded from regression analysis) are shown with an open, grey circle. Note: see Supplementary Fig. 4 for equivalent T_{crit} TSM values assuming that leaf T exceeds air T by +5 and +10 °C, and Supplementary Fig. 5 for equivalent T_{max} TSM values assuming that leaf T = air T, and exceeds air T by +5 and +10 °C

Future warming impacts on thermal safety margins

At two sites (GWW and Atherton), we assessed the extent of seasonal acclimation of T_{crit} and T_{max} in three species at each site. By measuring HT_{tol} in the same species in cool and warm seasons, we could quantify the extent to which T_{crit} and T_{max} varied in response to changes in mean daily T of the 30-day period prior to measurements. Table S6 shows that at GWW (temperate woodland), T_{crit} and T_{max} increased by 0.30 and 0.35 °C per 1.0 °C difference in growth T, respectively, when averaged across the three selected species; at Atherton, T_{crit} and T_{max} increased on average by 0.49 and 0.18 °C per 1.0 °C difference in growth T, respectively. Thus, while not a definitive measure of thermal acclimation, the data point to seasonal adjustments consistent with HT_{tol} increasing as growth T increases.

Because of limited knowledge of the extent of thermal acclimation of T_{crit} and T_{max} across our all species and the 19 sites, we used the relationships with current climate in Fig. 2b and Table 3 to simulate T_{crit} and T_{max} increases with climate warming (see Methods), noting that both traits exhibit evidence of seasonal acclimation in the species shown in Table S6. Here, we assumed that the geographic patterns in HT_{tol} under current climates mirror that of local thermal acclimation or adaptation to a warming climate, as done for modelling leaf respiration rates (Atkin *et al.,* 2015; Slot & Kitjima, 2015; Vanderwel *et al.*, 2015). The observed relationship with climate indicates a ~0.3 °C difference in HT_{tol} per 1.0 °C difference in growth T (Fig. 2b), which is consistent with empirical evidence of Ghouil *et al*. (2003) and is similar to the seasonal shifts in HT_{tol} shown in Table S6; given this, we used relationships shown in Fig. 2b and Table 3 as a first-order approximation of acclimation of HT_{tol} parameters to warmer future growing conditions. Thus, the slopes of the relationships of T_{crit} and T_{max} against mean maximum T of the warmest month (Fig. 2b) were used to predict T_{crit} and T_{max} values of each species under warmer climates projected for sites by 2050 under the IPCC high emission RCP8.5 scenario. While T_{crit} and T_{max} may not thermally acclimate in all species, predicting future T_{crit} and T_{max} values in a warmer world in this way avoids the unlikely assumption that HT_{tol} will remain static.

357 Under the above described thermal acclimation scenario, HT_{tol} increases in all species with
358 climate warming. Despite this, heat-wave *T* extremes are likely to breach T_{crit} across a broader range of
359 latitudes than today under the RCP8.5 scenario (Fig. 3b), with a narrowing of thermal safety margins
360 across all sites by 2050 (Fig. 4b, Table 2), due to the predicted increases in air *T* being greater than the
361 predicted acclimation-dependent increases in T_{crit} (for full comparison of different RCP scenarios, see
362 Table S3). When averaged across all sites and assuming leaf and air *T* are in equilibrium, 5% and 7% of
363 species are likely to exceed their T_{crit} thermal safety margins in current and future climates, respectively.
364 Importantly, the degree of narrowing of the thermal safety margin in 2050 would be even greater for
365 species that do not acclimate much or at all. Thus, by assuming a uniform degree of acclimation for all
366 species, our approach provides a conservative estimate of the possible effects of future warming on the
367 thermal safety margin, noting that the deleterious effects of warming would be even more severe if HT_{tol}
368 fails to increase proportionally as growth *T* increases above an optimum, as has been suggested (Barua
369 *et al.,* 2004, 2008; Wang *et al.,* 2008).

370 While future thermal safety margins are influenced by metabolic acclimation, consideration of
371 the extent to which leaf *T*s exceed air *T*s is also important. The above analyses assumed that leaf *T*s are
372 in equilibrium with air *T*, with the latter being based on climate station *T*s. Although leaf *T*s may match
373 ambient air *T*s in the shade, they can become markedly elevated during periods of full sun (Vogel, 2009;
374 Leigh *et al*., 2012), dependent on several factors including the extent of air movement, degree of
375 evaporative cooling and leaf shape. Leaf *T*s have been recorded to be 5-15 °C and 5-20 °C above ambient
376 air *T* in temperate and tropical regions, respectively (Table S1) at times of full sun, low wind speed and
377 reduced transpiration - conditions likely to co-occur during heat-waves. An elevated leaf *T* that is 5-10
378 °C above ambient air *T* across all sites would result in 18-43% of the sampled species exceeding their
379 thermal safety margin of T_{crit} based on recent heat-wave events (2001-2010; Table 2 & Fig. S4a,c), with
380 the proportion rising to 28-62% by 2050 under the RCP8.5 scenario (Table 2 & Fig. S4b,d). Thermal

safety margins calculated from T_{max}, reflecting tissue damage and death, are much less likely to be breached than thermal safety margins calculated from T_{crit} under current and future conditions (Fig. S5 & Table S4). The majority of species exceeding their thermal safety margins for T_{crit} occur at mid-latitude sites that currently experience the warmest heat-waves (2001-2010) with 100% of measured species at several sites exceeding their thermal safety margin in some climate change scenarios (Table S3). The mid-latitudes are also most likely to experience the warmest extremes in the future and to have the greatest fraction of species at risk of damage from such events (Table 2).

Discussion

Biogeographic patterns and acclimation/adaptation

Our results show that site mean values of observed T_{crit} and T_{max} of upper canopy leaves increase as site maximum air *T* increases (Fig. 2), although by less than half as much as the change in air *T* (Table 1, Fig. S1), resulting in a narrowing of the thermal safety margin in the mid-latitudes compared to the equatorial and high-latitude regions (Fig. 4; Figs S4 & S5). Importantly, thermal safety margins shown in Figure 4 likely over-estimate the true safety margin of upper canopy leaves as they assume that leaf and air *T*s are in equilibrium; during heat-waves, leaf *T*s are increasingly likely to exceed air *T*s, further reducing the safety margin (Figs S4 and S5). Thus, the extent to which upper canopy leaves exhibit *T*s greater than that of the surrounding air is crucial in determining how current and future heat-waves events impact on leaf metabolic processes central to plant growth and survival. A further factor that may contribute to the thermal safety margins shown for the 'Future (RCP 8.5)' scenarios shown in Figures 4, S4 and S5 is the assumption that HT_{tol} increases linearly with increasing growth *T* without an upper acclimation limit. Limits to the extent to which HT_{tol} can acclimate (Barua *et al.*, 2003, 2004; Krause *et al.* 2013) would further limit thermal safety margins of leaf energy metabolism in a future, warmer world.

405 The greater HT_{tol} in hot, low-mid latitude regions might simply reflect phenotypic adjustments of
406 individual plants to sustained exposure to high air *T* (i.e. thermal acclimation). Indeed, at two field sites,
407 we have observed seasonal adjustments in several species consistent with thermal acclimation of HT_{tol}
408 (Table S6). Acclimation to high air *T*s is underpinned by: changes in lipid composition and/or
409 accumulation of volatile organic compounds that increase membrane thermostability; increases in leaf
410 osmotic potential and soluble sugar concentrations that help protect chloroplast and mitochondrial
411 membranes; induction of heat shock proteins (HSPs) which act as molecular chaperones to protect
412 proteins from denaturation; and, accumulation of antioxidant enzymes to limit formation of reactive
413 oxygen species (Björkman *et al.*, 1980; Seemann *et al.*, 1986; Downs *et al.,* 1998; Heckathorn *et al.;*
414 1998; Sung *et al.*, 2003; Larkindale *et al.*, 2005; Sharkey, 2005; Hüve *et al.*, 2006, 2011; Velikova *et al.*,
415 2011; Kim *et al.,* 2012). Acclimation can allow the plant to cope, up to a point (that is yet to be
416 quantified), with extreme heat-wave events in a manner analogous to the way acclimation induced by
417 sustained chilling allows a plant to cope with a freezing event (Iba, 2002). Notwithstanding evidence of
418 seasonal adjustments in HT_{tol} at two of our field sites (Table S6), the extent to which T_{crit} and T_{max} adjust
419 to changes in growth *T per se* is not certain. For example, Krause *et al.* (2013) reported no change in
420 HT_{tol} of photosynthesis in tropical trees grown at two contrasting *T*s. Moreover, elevated growth *T* (3-5
421 °C) did not increase T_{max} of an Australian broadleaved tree, *Eucalyptus saligna* (Gauthier *et al*., 2014) or
422 an arctic shrub, *Betula nana* (Heskel *et al.*, 2014); similarly, increases in growth *T* along an 800 m
423 elevational gradient in the sub-alpine/alpine region of SE Australia had no effect on either T_{crit} or T_{max} of
424 *Eucalyptus pauciflora* (O'Sullivan *et al*., 2013). By contrast, Ghouil *et al*. (2003) reported that when cork
425 oak (*Quercus suber*) plants were acclimated to a wider range of growth *T*s (10-40°C), T_{crit} increased by
426 ~0.3 °C per 1.0°C increase in growth *T*. Acclimation of T_{crit} and related photosynthetic properties has
427 also been reported in several studies (Downton *et al.,* 1984; Seemann *et al.,* 1986; Knight & Ackerly,
428 2002). Thus, while the extent to which T_{crit} and T_{max} acclimates remains unclear, the possibility remains

429 that acclimation may contribute to the biogeographical patterns observed in our study, particularly given
430 our observation of seasonal adjustments in T_{crit} and T_{max} (Table S6). While further work is needed to
431 assess the extent to which T_{crit} and T_{max} acclimate to sustained changes in *T per se*, a conservative
432 approach to predicting potential negative effects of future warming would assume that acclimation does
433 occur – it is for this reason that we assumed a uniform level of acclimation for all our selected species
434 when modelling the effect of future (RCP 8.5) warming on the thermal safety margin of HT_{tol} (Fig. 4b
435 and Figs S5 & S5). For our analyses, we assumed that the slope of relationships linking T_{max} and T_{crit} to
436 mean maximum *T* of the warmest month (Fig. 3b) could provide an estimate of acclimation potential,
437 with the assumed acclimation equations allowing for 0.38°C and 0.26°C change in T_{crit} and T_{max}
438 respectively per 1.0°C change in mean maximum *T* of the warmest month (see Table 3).
439 A second factor that may contribute to greater HT_{tol} in plants in hot regions is that heat tolerance
440 is a consequence of evolutionary history, whereby adaptation to hot climates results in inherently greater
441 HT_{tol} compared to plants adapted cooler regions. Notwithstanding evidence that both T_{crit} and T_{max} likely
442 acclimate to changes in *T* of the growth environment (see above), it remains unknown the extent to which
443 species from thermally contrasting environments differ in basal HT_{tol} and/or ability to increase HT_{tol}
444 when challenged with rising growth *T*s [but see related studies by Knight & Ackerly (2002) & Curtis *et*
445 *al.* (2014)]. Comparison of a diverse range of plant taxa from thermally contrasting biomes, when grown
446 under common garden conditions, are therefore needed to gain insights into the role acclimation versus
447 adaptation processes play in controlling the observed site-to-site patterns in HT_{tol} observed in our study.
448
449 *Upper limits to thermal tolerance*
450 The likelihood that there is an upper limit in HT_{tol} of photosynthetic and respiratory metabolism in
451 terrestrial plants – as has been suggested previously by studies focussing on the induction of chloroplast
452 and/or mitochondrial HSPs (Heckathorn *et al.;* 1998; Downs *et al.,* 1998; Shakeel *et al.,* 2011; Kim *et*

453 *al.,* 2012) and associated HT_{tol} of photosynthetic rates (Barua *et al.,* 2004, 2008; Krause *et al*., 2013) - is
454 reflected in the observation that maximum heat-wave air *T*s are not matched by concomitant increases in
455 HT_{tol} (hence the fact that the thermal safety margin of HT_{tol} is not constant – Fig. 4a; Figs S4 & S5).
456 While the above mentioned acclimation mechanisms are likely to lead to increased heat tolerance, it
457 seems likely that there is limit in the extent to which they facilitate increases in HT_{tol} of photosynthesis
458 and respiration, which in turn reduces the thermal safety margins of these processes for plants growing
459 at the warmest sites. Further work will be needed to quantify the upper limits of thermal tolerance of
460 photosynthesis and respiration in wide range of plant taxa from thermally contrasting biomes, particularly
461 in plants acclimated to high growth *T*s.
462
463 *Thermal safety margin*
464 A key factor that will influence changing thermal safety margins in plants is the extent to which other
465 driving variables modify how much leaf *T*s exceed air *T*s during heat-waves. The elevation of leaf *T*
466 above air *T* will be greatest under high irradiance and low soil moisture conditions that reduce stomatal
467 conductance, transpirational water loss, and associated cooling (Ameye *et al.,* 2012; Bauweraerts *et al.,*
468 2013, 2014; Schymanski *et al.*, 2013). Although there are reports of drought-mediated increases of 6-10
469 °C in T_{max} (Gauthier *et al.*, 2014) and T_{crit} (Ghouil *et al.*, 2003), loss of transpirational cooling during
470 drought events could result in even greater increases in leaf *T*s during heat-waves. Similarly, elevated
471 atmospheric CO_2 concentrations may also modestly increase leaf *T* (e.g. 2-5 °C) in both C_3 (Barker *et*
472 *al.*, 2005) and C_4 (Siebke *et al.*, 2002) plants via reduced stomatal conductance to water vapour and latent
473 heat loss. Such increases in leaf *T* could further narrow future thermal safety margins (Fig. 4b; Figs S4
474 & S5), depending on the extent to which rising atmospheric CO_2 concentrations impact on high *T*
475 tolerance (Ameye *et al.,* 2012). There is also the possibility that T_{crit} could rise in response to future
476 increases atmospheric CO_2, as a review by Wang *et al.* (2012) found increased heat tolerance of

photosynthesis in a range of C_3 species grown under elevated CO_2. However, past studies of heat stress and elevated CO_2 (e.g. Faria *et al.*, 1996; Taub *et al.*, 2000) have not quantified changes in T_{crit} *per se*. In the one study to assess the effects of elevated atmospheric CO_2 on T_{max} (Gauthier *et al.*, 2014), elevated CO_2 had no effect.

What would be the impacts of exceeding heat tolerance levels? For leaves transiently exposed (i.e. < 5 mins) to *T*s between T_{crit} and T_{max}, metabolic functioning continues at an impaired level (O'Sullivan *et al*., 2013). However, with more prolonged exposure to such leaf *T*s during heat-waves, leaf tissues will be permanently damaged (O'Sullivan *et al.*, 2013; Schymanski *et al.*, 2013). Thus, tissue death could occur during multi-day heat-wave events, with the ultimate impact depending on the extent to which leaves increase HT_{tol} during those events, combined with the extent to which embolisms and associated water stress increase during heat-waves. Further factors that may influence the impact of such heat-wave events on plant growth and survival is whether leaf metabolism can rapidly recover from heat stress (Curtis *et al.,* 2014), the extent to which lower canopy leaves maintain functionality and/or the speed with which upper canopy leaves re-grow following each heat-wave.

Variability in heat tolerance among co-occurring species

As reported in previous studies assessing heat tolerance of photosynthesis of field-grown plants (Knight & Ackerly, 2002, 2003; Curtis *et al.,* 2014), we found that T_{crit} and T_{max} were highly variable among co-occurring species at each site (Fig. 3). Thus, while many species can tolerate high leaf *T*s, other co-occurring species possess a smaller safety margin (i.e. lower T_{crit} and T_{max}) (Fig. 4 and Fig. S4). To gain insights into what factors might be responsible for this variability, we analysed whether including leaf structural and chemical composition traits that are ecologically important and linked to growth potential (SLA, [N] and [P] (Reich *et al.,* 1997; Wright *et al.,* 2004; Poorter *et al.,* 2009) improve our ability to predict variability in HT_{tol}. For T_{crit}, inclusion of SLA, [N] and [P] did not improve the predictive power

501 of the models (Table S5), irrespective of whether site-mean or species-mean data were used in the
502 analysis. While the results in Table 3 and S5 point to T_{crit} being linked to the thermal environment in
503 which plants are grown (either as a result of acclimation and/or adaptive processes), they do not explain
504 why co-existing species differed in T_{crit} values. Rather, other factors must be responsible for the observed
505 variability in T_{crit} among species at each site, such as variability in the abundance of heat shock proteins
506 (Heckathorn *et al*., 1998; Knight & Ackerly, 2001, 2003; Barua *et al*., 2003; 2008) and/or membrane
507 composition traits (Raison *et al.* 1982; Larkindale & Huang, 2004; Los & Murata, 2004).
508 Interestingly, leaf [P] was retained in the regression model predicting variability in T_{max} of
509 respiration when analyses were conducted using site-mean and species-mean values, with higher leaf [P]
510 being associated with decreased T_{max} (Tables 3 and S5). Thus, while membrane properties and heat shock
511 proteins (particularly proteins that accumulate in mitochondria; Downs *et al*., 1998; Kim *et al.,* 2012) are
512 likely to be important for species variability in T_{max}, it appears that processes linked to foliar phosphorus
513 concentrations influence species-to-species variation in heat tolerance of mitochondrial respiration;
514 further work is needed to explore the mechanistic basis via which this association occurs.
515
516 **Conclusions**
517 Taken together, our results indicate that upper canopy leaves of many plants in mid-latitudes operate
518 close to their metabolic thermal limits during heat-wave events (Figs 2 and 4). Leaves in such areas are
519 particularly at risk considering that air *T* extremes are highest in the mid-latitudes. Dry summers are
520 characteristic of the climate in large portions of this latitudinal zone, and if coupled with reduced
521 transpiration would further elevate leaf *T* above air *T*s. Moreover, heat-wave events will generally
522 become more common in the future. As mean air *T* in the tropics and mid-latitudes is predicted to exceed
523 current extreme *T* events by 2100 (Battisti & Naylor, 2009), vulnerability of upper canopy leaf
524 metabolism to heat-wave events will increasingly become a reality (Fig. 2b and Table 2; Figs S4 & S5

525 and Tables S4 & S5). Under future climate warming scenarios, our findings suggest that upper canopy
526 leaf metabolism may be at substantially increased risk during heat-wave events, particularly when those
527 are combined with drought (IPCC, 2012). Although this finding is speculative, it is worth noting that
528 native plants in mid-latitude regions may be particularly at risk. If loss of metabolic capacity impacts on
529 rates of net carbon gain at the whole-plant level, there is a possibility that heat-waves may contribute to
530 dieback of heat-sensitive species, with consequences for gross primary production (GPP) and global
531 species distributions in a future, warmer world (Reyer *et al.*, 2013). Finally, when considering the extent
532 to which heat-waves will impact on GPP and distribution of plant species, an assessment is needed not
533 just of HT_{tol} of leaf metabolism, but also how heat affects other plant processes during vegetative growth
534 (e.g. efficiency of water use) and reproduction, with the latter being particularly sensitive to heat-waves
535 (Barnabas *et al*., 2008; Hall, 2010).
536
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550

551 **References**

552

553 Allen SE (1974) *Chemical analysis of ecological materials,* Oxford, Blackwell Scientific Publications.

554 Ansari AQ, Loomis WE (1959) Leaf temperatures. *American Journal of Botany,* **46**, 713-717.

555 Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL (2013) Heat freezes
556 niche evolution. *Ecology Letters,* **16**, 1206-1219.

557 Ameye M, Wertin TM, Bauweraerts I, McGuire MA, Teskey RO, Steppe K (2012) The effect of induced
558 heat waves on *Pinus taeda* and *Quercus rubra* seedlings in ambient and elevated CO_2
559 atmospheres. *New Phytologist,* **196**, 448-461.

560 Atkin OK, Bloomfield KJ, Reich PB *et al.* (2015) Global variability in leaf respiration in relation to
561 climate, plant functional types and leaf traits. *New Phytologist,* **206**, 614-636.

562 Atkin OK, Scheurwater I, Pons TL (2006) High thermal acclimation potential of both photosynthesis and
563 respiration in two lowland *Plantago* species in contrast to an alpine congeneric. *Global Change*
564 *Biology,* **12**, 500-515.

565 Barker DH, Loveys BR, Egerton JJG, Gorton H, Williams WE, Ball MC (2005) CO_2 enrichment
566 predisposes foliage of a eucalypt to freezing injury and reduces spring growth. *Plant, Cell &*
567 *Environment,* **28**, 1506-1515.

568 Barnabas B, Jager K, Feher A (2008) The effect of drought and heat stress on reproductive processes in
569 cereals. *Plant Cell & Environment,* **31,** 11-38.

570 Barua D, Downs CA, Heckathorn SA (2003) Variation in chloroplast small heat-shock protein function
571 is a major determinant of variation in thermotolerance of photosynthetic electron transport among
572 ecotypes of *Chenopodium album*. *Functional Plant Biology,* **30,** 1071-1079.

573 Barua D, Heckathorn SA (2004) Acclimation of the temperature set-points of the heat-shock response.
574 *Journal of Thermal Biology,* **29**, 185-193.

575 Barua D, Heckathorn SA, Coleman JS (2008) Variation in heat-shock proteins and photosynthetic
576 thermotolerance among natural populations of *Chenopodium album* L. from contrasting thermal
577 environments: implications for plant responses to global warming. *Journal of Integrative Plant*
578 *Biology,* **50**, 1440-1451.

579 Battisti DS, Naylor RL (2009) Historical warnings of future food insecurity with unprecedented seasonal
580 heat. *Science,* **323**, 240-244.

581 Bauweraerts I, Ameye M, Wertin TM, McGuire MA, Teskey RO, Steppe K (2014) Water availability is
582 the decisive factor for the growth of two tree species in the occurrence of consecutive heat waves.
583 *Agricultural and Forest Meteorology,* **189–190**, 19-29.
584 Bauweraerts I, Wertin TM, Ameye M, McGuire MA, Teskey RO, Steppe K (2013) The effect of heat
585 waves, elevated [CO_2] and low soil water availability on northern red oak (*Quercus rubra* L.)
586 seedlings. *Global Change Biology,* **19**, 517-528.
587 Beadle CL, Stevenson KR, Thurtell GW (1973) Leaf temperature measurement and control in a gas-
588 exchange cuvette. *Canadian Journal of Plant Science,* **53**, 407-412.
589 Berry JA, Björkman O (1980) Photosynthetic response and adaptation to temperature in higher plants.
590 *Annual Review of Plant Physiology,* **31**, 491-543.
591 Björkman O, Badger M, Armond PA, Turner NC, Kramer PJ (1980) Response and adaptation of
592 photosynthesis to high temperatures. In: *Adaptation of Plants to Water and High Temperature*
593 *Stress.* pp Page. New York, John Wiley and Sons.
594 Curtis E, Knight C, Petrou K, Leigh A (2014) A comparative analysis of photosynthetic recovery from
595 thermal stress: a desert plant case study. *Oecologia,* **175,** 1051-1061.
596 Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts
597 of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National*
598 *Academy of Sciences,* USA, **105**, 6668-6672.
599 Dewar RC, Medlyn BE, Mcmurtrie RE (1999) Acclimation of the respiration/photosynthesis ratio to
600 temperature: insights from a model. *Global Change Biology,* **5**, 615-622.
601 Doughty CE, Goulden ML (2008) Are tropical forests near a high temperature threshold? *Journal of*
602 *Geophysical Research: Biogeosciences,* **113**, G00B07.
603 Downs CA, Heckathorn SA (1998) The mitochondrial small heat-shock protein protects
604 NADH:ubiquinone oxidoreductase of the electron transport chain during heat stress in plants.
605 *Febs Letters,* **430,** 246-250.
606 Downton WJS, Berry JA, Seemann JR (1984) Tolerance of photosynthesis to high-temperature in desert
607 plants. *Plant Physiology,* **74,** 786-790.
608 Faria T, Wilkins D, Besford RT, Vaz M, Pereira JS, Chaves MM (1996) Growth at elevated CO_2 leads
609 to down-regulation of photosynthesis and altered response to high temperature in *Quercus suber*
610 L seedlings. *Journal of Experimental Botany,* **47**, 1755-1761.
611 Friedlingstein P, Andrew RM, Rogelj J *et al.* (2014) Persistent growth of CO_2 emissions and implications
612 for reaching climate targets. *Nature Geoscience,* **7**, 709-715.

613 Fuchs M (1990) Infrared measurement of canopy temperature and detection of plant water stress.
614 *Theoretical and Applied Climatology,* **42**, 253-261.
615 Ganguly AR, Steinhaeuser K, Erickson DJ *et al.* (2009) Higher trends but larger uncertainty and
616 geographic variability in 21st century temperature and heat waves. *Proceedings of the National*
617 *Academy of Sciences, USA,* **106**, 15555-15559.
618 Gates DM, Alderfer R, Taylor E (1968) Leaf temperatures of desert plants. *Science,* **159**, 994-995.
619 Gauthier PPG, Crous KY, Ayub G *et al.* (2014) Drought increases heat tolerance of leaf respiration in
620 *Eucalyptus globulus* saplings grown under ambient and elevated atmospheric [CO_2] and
621 temperature. *Journal of Experimental Botany,* **65**, 6471–6485.
622 Ghouil H, Montpied P, Epron D, Ksontini M, Hanchi B, Dreyer E (2003) Thermal optima of
623 photosynthetic functions and thermostability of photochemistry in cork oak seedlings. *Tree*
624 *Physiology,* **23**, 1031-1039.
625 Givnish T (1988) Adaptation to sun and shade: a whole-plant perspective. *Functional Plant Biology,* **15**,
626 63-92.
627 Hall AE (2010) Breeding for Heat Tolerance. *Plant Breeding Reviews*, pp. 129-168. John Wiley & Sons,
628 Inc.
629 Hansen J, Sato M, Ruedy R (2012) Perception of climate change. *Proceedings of the National Academy*
630 *of Sciences, USA,* **109**, E2415-E2423.
631 Hazel JR (1995) Thermal adaptation in biological membranes: is homeoviscous adaptation the
632 explanation? *Annual Review of Physiology,* **57**, 19-42.
633 Heckathorn SA, Downs CA, Sharkey TD, Coleman JS (1998) The small, methionine-rich chloroplast
634 heat-shock protein protects photosystem II electron transport during heat stress. *Plant Physiology,*
635 **116,** 439-444.
636 Heskel MA, Greaves HE, Turnbull MH, O'Sullivan OS, Shaver GR, Griffin KL, Atkin OK (2014)
637 Thermal acclimation of shoot respiration in an Arctic woody plant species subjected to 22 years
638 of warming and altered nutrient supply. *Global Change Biology,* **20**, 2618–2630.
639 Heskel MA, O'Sullivan OS, Reich PB *et al.* (2016) Convergence in the temperature response of leaf
640 respiration across biomes and plant functional types. *Proceedings of the National Academy of*
641 *Sciences,* USA, **113**, 3832-3837.
642 Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate
643 surfaces for global land areas. *International Journal of Climatology,* **25**, 1965-1978.

644 Hochachka PW, Somero GN (2002) *Biochemical adaptation: Mechanism and process in physiological*
645 *evolution,* New York, NY, Oxford University Press, Inc. .
646 Hüve K, Bichele I, Ivanova H *et al.* (2012) Temperature responses of dark respiration in relation to leaf
647 sugar concentration. *Physiologia Plantarum,* **144**, 320-334.
648 Hüve K, Bichele I, Rasulov B, Ninemets Ü (2011) When it is too hot for photosynthesis: heat-induced
649 instability of photosynthesis in relation to respiratory burst, cell permeability changes and H_2O_2
650 formation. *Plant Cell and Environment,* **34**, 113-126.
651 Hüve K, Bichele I, Tobias M, Niinemets Ü (2006) Heat sensitivity of photosynthetic electron transport
652 varies during the day due to changes in sugars and osmotic potential. *Plant Cell and Environment,*
653 **29**, 212-228.
654 Iba K (2002) Acclimative response to temperature stress in higher plants: Approaches of gene
655 engineering for temperature tolerance. *Annual Review of Plant Biology,* **53**, 225-245.
656 IPCC (2012) *Managing the Risks of Extreme Events and Disasters to Advance Climate Change*
657 *Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on*
658 *Climate Change,* Cambridge, UK and New York, NY, USA, Cambridge University Press.
659 Ishida A, Toma T, Marjenah (1999) Limitation of leaf carbon gain by stomatal and photochemical
660 processes in the top canopy of *Macaranga conifera*, a tropical pioneer tree. *Tree Physiology,* **19**,
661 467-473.
662 Kim M, Lee U, Small I, des Francs-Small CC, Vierling E. (2012) Mutations in an *Arabidopsis*
663 mitochondrial transcription termination factor–related protein enhance thermotolerance in the
664 absence of the major molecular chaperone *HSP101*. *The Plant Cell,* **24,** 3349-3365.
665 Knight CA, Ackerly DD (2001) Correlated evolution of chloroplast heat shock protein expression in
666 closely related plant species. *American Journal of Botany,* **88,** 411-418.
667 Knight CA, Ackerly DD (2002) An ecological and evolutionary analysis of photosynthetic
668 thermotolerance using the temperature-dependent increase in fluorescence. *Oecologia,* **130**, 505-
669 514.
670 Knight CA, Ackerly DD (2003) Evolution and plasticity of photosynthetic thermal tolerance, specific
671 leaf area and leaf size: congeneric species from desert and coastal environments. *New Phytologist,*
672 **160,** 337-347.
673 Krause GH, Cheesman AW, Winte, K, Krause B, Virgo A (2013) Thermal tolerance, net CO_2 exchange
674 and growth of a tropical tree species, *Ficus insipida*, cultivated at elevated daytime and nighttime
675 temperatures. *Journal of Plant Physiology,* **170,** 822-827.

676 Larkindale J, Huang B (2004) Changes of lipid composition and saturation level in leaves and roots for
677 heat-stressed and heat-acclimated creeping bentgrass (*Agrostis stolonifera*). *Environmental and*
678 *Experimental Botany,* **51,** 57-67.
679 Larkindale J, Jennifer D. Hall, Knight MR, Vierling E (2005) Heat stress phenotypes of Arabidopsis
680 mutants implicate multiple signaling pathways in the acquisition of thermotolerance. *Plant*
681 *Physiology,* **138**, 882-897.
682 Leigh A, Sevanto S, Ball MC *et al.* (2012) Do thick leaves avoid thermal damage in critically low wind
683 speeds? *New Phytologist,* **194**, 477-487.
684 Leuzinger S, Vogt R, Körner C (2010) Tree surface temperature in an urban environment. *Agricultural*
685 *and Forest Meteorology,* **150**, 56-62.
686 Los DA, Murata N (2004) Membrane fluidity and its roles in the perception of environmental signals.
687 *Biochimica et Biophysica Acta (BBA) - Biomembranes,* **1666,** 142-157.
688 Luquet D, Bégué A, Vidal A *et al.* (2003) Using multidirectional thermography to characterize water
689 status of cotton. *Remote Sensing of Environment,* **84**, 411-421.
690 Miller PC (1972) Bioclimate, leaf temperature, and primary production in red mangrove canopies in
691 south Florida. *Ecology,* **53**, 22-45.
692 Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL, Tsukaya H (2011) The evolution and
693 functional significance of leaf shape in the angiosperms. *Functional Plant Biology,* **38**, 535-552.
694 O'Sullivan OS, Weerasinghe KWLK, Evans JR, Egerton JJG, Tjoelker MG, Atkin OK (2013) High-
695 resolution temperature responses of leaf respiration in snow gum (*Eucalyptus pauciflora*) reveal
696 high-temperature limits to respiratory function. *Plant, Cell & Environment,* **36**, 1268-1284.
697 Perkins S, Alexander L, Nairn J (2012) Increasing frequency, intensity and duration of observed global
698 heatwaves and warm spells. *Geophysical Research Letters,* **39**, L20714.
699 Poorter H, Niinemets U, Poorter L *et al.* (2009) Causes and consequences of variation in leaf mass per
700 area (LMA): a meta-analysis. *New Phytologist,* **182,** 565-588.
701 Raison JK, Roberts JKM, Berry JA (1982) Correlations between the thermal stability of chloroplast
702 (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of
703 the higher plant, *Nerium oleander*, to growth temperature. *Biochimica et Biophysica Acta (BBA) -*
704 *Biomembranes,* **688,** 218-228.
705 Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: Global convergence in plant
706 functioning. *Proceedings of the National Academy of Sciences, USA,* **94,** 13730-13734.

Reyer CPO, Leuzinger S, Rammig A *et al.* (2013) A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. *Global Change Biology,* **19**, 75-89.

Schreiber U, Berry J (1977) Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. *Planta,* **136**, 233-238.

Schymanski S, Or D, Zwieniecki M (2013) Stomatal control and leaf thermal and hydraulic capacitances under rapid environmental fluctuations. *PLoS ONE,* **8**, e54231.

Seemann JR, Berry JA, Downton WJS (1984) Photosynthetic response and adaptation to high-temperature in desert plants - a comparison of gas-exchange and fluorescence methods for studies of thermal tolerance. *Plant Physiology,* **75**, 364-368.

Seemann JR, Downton WJS, Berry JA (1986) Temperature and leaf osmotic potential as factors in the acclimation of photosynthesis to high-temperature in desert plants. *Plant Physiology,* **80**, 926-930.

Shakeel S, Haq NU, Heckathorn SA, Hamilton EW, Luthe, DS (2011) Ecotypic variation in chloroplast small heat-shock proteins and related thermotolerance in *Chenopodium album*. *Plant Physiology & Biochemistry*, 49, 898-908.

Sharkey TD (2005) Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant, Cell and Environment,* **28**, 269-277.

Siebke K, Ghannoum O, Conroy JP, Caemmerer SV (2002) Elevated CO_2 increases the leaf temperature of two glasshouse-grown C_4 grasses. *Functional Plant Biology,* **29**, 1377-1385.

Singsaas EL, Sharkey TD (1998) The regulation of isoprene emission responses to rapid leaf temperature fluctuations. *Plant, Cell & Environment,* **21**, 1181-1188.

Slot M, Kitajima K (2015) General patterns of acclimation of leaf respiration to elevated temperatures across biomes and plant types. *Oecologia,* **177**, 885-900.

Smith WK (1978) Temperatures of desert plants: another perspective on the adaptability of leaf size. *Science,* **201**, 614-616.

Strahler AN, Strahler AH (1989) *Elements of Physical Geography*, 4th ed., John Wiley & Sons.

Sunday JM, Bates AE, Dulvy NK (2011) Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences,* **278**, 1823-1830.

Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB (2014) Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences USA,* **111**, 5610-5615.

739 Sung D-Y, Kaplan F, Lee K-J, Guy CL (2003) Acquired tolerance to temperature extremes. *Trends in*
740 *Plant Science,* **8**, 179-187.
741 Taub DR, Seemann JR, Coleman JS (2000) Growth in elevated CO_2 protects photosynthesis against high-
742 temperature damage. *Plant Cell and Environment,* **23**, 649-656.
743 Teskey R, Wertin T, Bauweraerts I, Ameye M, McGuire MA, Steppe K (2014) Responses of tree species
744 to heat waves and extreme heat events. *Plant, Cell & Environment,* **38**, 1699-1712.
745 Trubuzi ES (2005) Variações da temperatura foliar do dossel e o seu efeito na taxa assimilatória de CO_2
746 na Amazônia Central. PhD, Universidade de São Paulo, São Paulo, 84 pp.
747 Tyree MT, Wilmot TR (1990) Errors in the calculation of evaporation and leaf conductance in steady-
748 state porometry: the importance of accurate measurement of leaf temperature. *Canadian Journal*
749 *of Forest Research,* **20**, 1031-1035.
750 UNEP (1997) *World Atlas of Desertification,* London, United Nations Environment Programme.
751 Valladares F, Pearcy RW (1997) Interactions between water stress, sun-shade acclimation, heat tolerance
752 and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. *Plant Cell & Environment,* **20**,
753 25-36.
754 Vanderwel MC, Slot M, Lichstein JW *et al.* (2015) Global convergence in leaf respiration from estimates
755 of thermal acclimation across time and space. *New Phytologist,* **207**, 1026-1037.
756 Velikova V, Várkonyi Z, Szabó M *et al.* (2011) Increased thermostability of thylakoid membranes in
757 isoprene-emitting leaves probed with three biophysical techniques. *Plant Physiology,* **157**, 905-
758 916.
759 Vogel S (2009) Leaves in the lowest and highest winds: temperature, force and shape. *New Phytologist,*
760 **183**, 13-26.
761 Vuuren D, Edmonds J, Kainuma M *et al.* (2011) The representative concentration pathways: an
762 overview. *Climatic Change,* **109**, 5-31.
763 Wang D, Heckathorn SA, Barua D *et al*. (2008) Effects of elevated CO_2 on the tolerance of
764 photosynthesis to acute heat stress in C_3, C_4, and CAM species. *American Journal of Botany,* **95,**
765 165-176.
766 Wang D, Heckathorn SA, Wang XZ, Philpott SM (2012) A meta-analysis of plant physiological and
767 growth responses to temperature and elevated CO_2. *Oecologia,* **169,** 1-13.
768 Weerasinghe LK, Creek D, Crous KY, Xiang S, Liddell MJ, Turnbull MH, Atkin OK (2014) Canopy
769 position affects the relationships between leaf respiration and associated traits in a tropical
770 rainforest in Far North Queensland. *Tree Physiology,* **34**, 564-584.

771 Wright IJ, Reich PB, Westoby M, *et al.* (2004) The worldwide leaf economics spectrum. *Nature,* **428,**
772 821-827.
773 Zomer RJ, Trabucco A, Bossio DA, Verchot LV (2008) Climate change mitigation: A spatial analysis of
774 global land suitability for clean development mechanism afforestation and reforestation.
775 *Agriculture, Ecosystems & Environment,* **126**, 67-80.
776 Zweifel R, Zimmermann L, Zeugin F, Newbery DM (2006) Intra-annual radial growth and water
777 relations of trees: implications towards a growth mechanism. *Journal of Experimental Botany,*
778 **57**, 1445-1459.
779

780 **Supporting Information**

781 Figure S1. Comparison of *WorldClim* predictions and observed values of maximum temperatures.
782 Figure S2. Re-analysis of Figure 2 in main text, revealing hemisphere-specific responses of high *T*
783 tolerance
784 Figure S3. Re-analysis of Figure 2 in the main text, revealing growth habit specific responses of high *T*
785 tolerance
786 Figure S4. Thermal safety margin values of T_{crit} for scenarios where leaf *T* is +5 and +10°C > air *T*.
787 Figure S5. Thermal safety margin values of T_{max} for scenarios where leaf *T* is in equilibrium with air *T*,
788 and where leaf *T* is +5 and +10°C > air *T*.
789 Table S1. Maximum increase in leaf *T* above air temperatures as reported in literature
790 Table S2. Details of nearest meteorological stations to each of the sites.
791 Table S3. Percentage of species where thermal safety margin (T_{crit}) was exceeded under different climate
792 scenarios
793 Table S4. Percentage of species where thermal safety margin (T_{max}) was exceeded under different climate
794 scenarios
795 Table S5. Regression equations expressing T_{max} and T_{crit} as a function of climate and other leaf traits,
796 using species mean data on leaf traits.
797 Table S6 Seasonal changes in in high *T* tolerance T_{max} and T_{crit} at two field sites in Australia
798 Table S7. Source data (Excel file).

Supporting Information

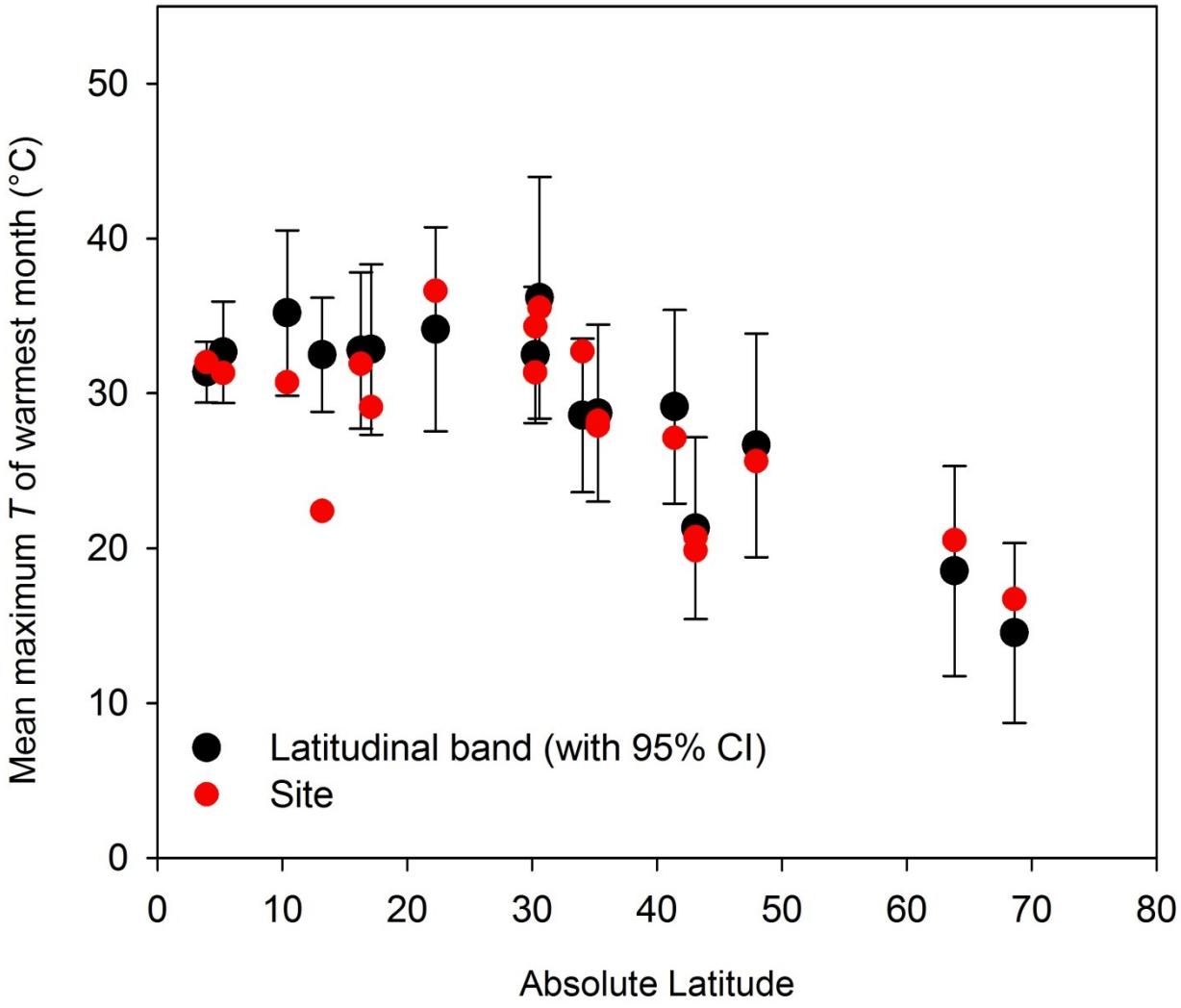

Fig. S1 Comparison of *WorldClim* database predictions of mean maximum daily temperature (*T*) of the warmest month for each site (red circles), and mean maximum daily *T* of the warmest month of the respective 1° latitudinal band (land only) for each site (black circles); for the latter, error bars show 95% confidence interval for each latitudinal band. All sites are within this band with the exception of the high altitude Andes site in Peru (at 13° latitude). These data confirm that the sampled sites are not outliers in relation to thermal environment with latitude (with the exception of the high altitude site in Peru).

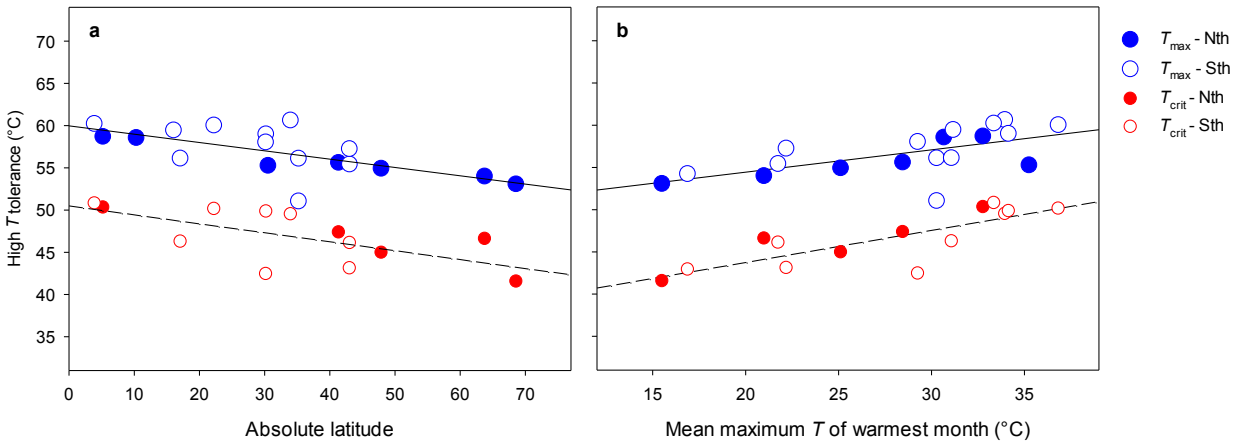

Fig. S2. Re-analysis of data of site means shown in Figure 2 in the main text, revealing hemisphere-specific responses of high *T* tolerance (T_{max} and T_{crit}) against: (a) absolute latitude; and (b) mean maximum *T*s of the warmest month (MTWM) at each site. Analysis of covariance (ANCOVA – using latitude or MTWM as the covariant) revealed no main effect of hemisphere or interaction between hemisphere and latitude/MTWM in either (a) or (b). The same was true when using species average data instead of site mean data. Thus, there is no evidence of the two hemispheres differing in high *T* tolerance at any given latitude or MTWM. Regression lines shown are for overall relationships combining both hemispheres together. Note: for analyses in (a), data from the high altitude Andean site in Peru were excluded from the regression and ANCOVA analyses.

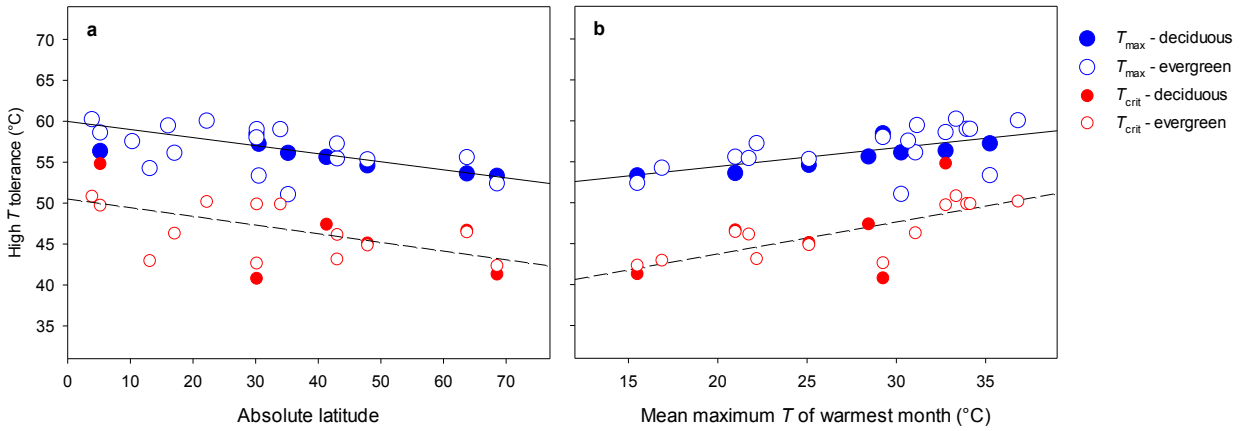

Fig. S3. Re-analysis of data shown in Figure 2 in the main text, revealing leaf growth habit (deciduous, evergreen) responses of high *T* tolerance (T_{max} and T_{crit}) against: (a) absolute latitude; and (b) mean maximum *T*s of the warmest month (MTWM) for deciduous and evergreen species means pooled at each site. Analysis of covariance (ANCOVA – using latitude or MTWM as the covariant) revealed no main effect of leaf growth habit or interaction between leaf growth habit and latitude/MTWM in either (a) or (b). The same was true when using species average data instead of site mean data. Thus, there is no evidence of evergreen and deciduous species differing in high *T* tolerance at any given latitude or MTWM. Regression lines shown are for overall relationships combining both growth habits together. Note: for analyses in (a), data from the high altitude Andean site in Peru were excluded from the regression and ANCOVA analyses.

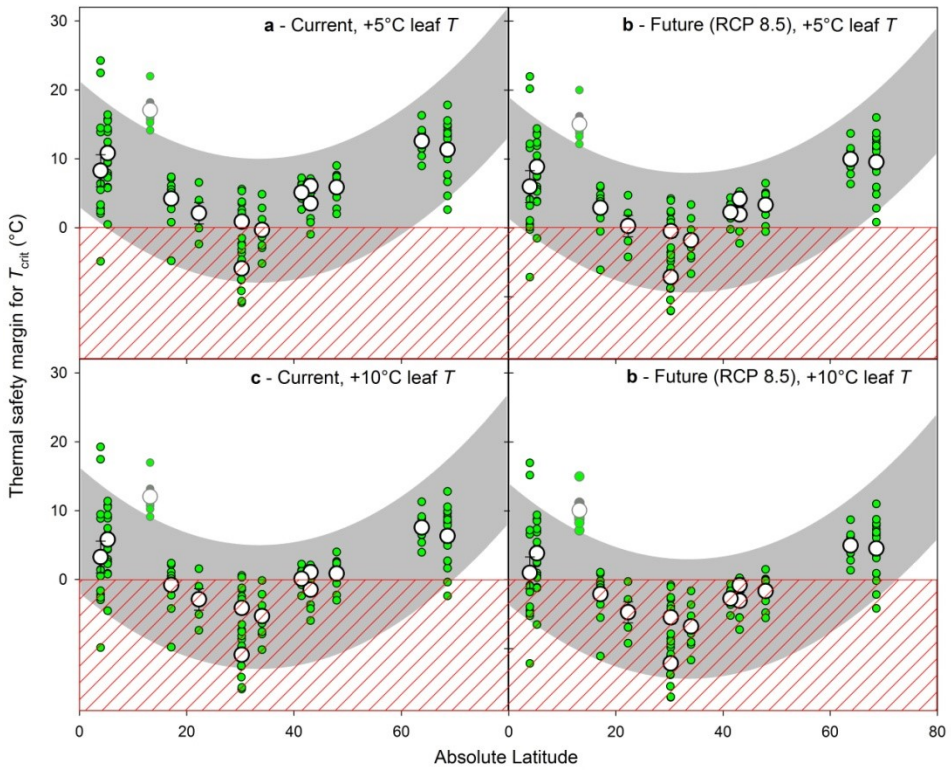

Fig. S4 Thermal safety margin values calculated as the difference between high *T* tolerance of photosynthesis (T_{crit}) and air temperature (*T*) in relation to absolute latitude, as determined using mean maximum temperature (*T*) over warmest consecutive 3-day period from 2001-2010 (**a,c**), and estimated future (RCP 8.5) mean maximum 3-day heat-wave *T*s (**b,d**), with maximum *T* based values used in Fig. 3 in the main text. Thermal safety margins shown for scenarios where leaf T exceeds air T by +5 °C (a,c) and +10 °C (b,d). Larger open circles (○) show site means ± SE of the thermal safety margin using T_{crit}. Site-mean values for a high altitude site in the Peruvian Andes at a latitude of 13°S (excluded from regression analysis) are shown with an open, grey circle. Species mean data are shown in smaller green circles behind site mean data. Grey shading shows 95% C.I. interval for T_{crit} thermal safety margin. Red hatched box indicates leaf injury zone at which T_{crit} has been exceeded (and thus there is no thermal safety margin). T_{crit} refers to the high *T* tolerance of photohesis, as defined in the main text.

Fig. S5 Thermal safety margin values calculated as the difference between high *T* tolerance of respiration (T_{max}) and air temperature (*T*) in relation to absolute latitude, as determined using mean maximum temperature (*T*) over warmest consecutive 3-day period from 2001-2010 (**a,c,e**), and estimated future (RCP 8.5) mean maximum 3-day heat-wave *T*s (**b,d,f**), with maximum *T* based values used in Fig. 3 in the main text. Thermal safety margins shown for scenarios where leaf *T* = air *T* (**a,b**), leaf *T* exceeds air *T* by +5 °C (**c,d**) and +10 °C (**e,f**). Larger open circles (○) show site means ± SE of the thermal safety margin using T_{max}. Site-mean values for a high altitude site in the Peruvian Andes at a latitude of 13°S (excluded from regression analysis) are shown with an open, grey circle. Species mean data are shown in smaller green circles behind site mean data. Grey shading shows 95% C.I. interval for T_{max} thermal safety margin. Red hatched box indicates leaf injury zone at which T_{max} has been exceeded (and thus there is no thermal safety margin).

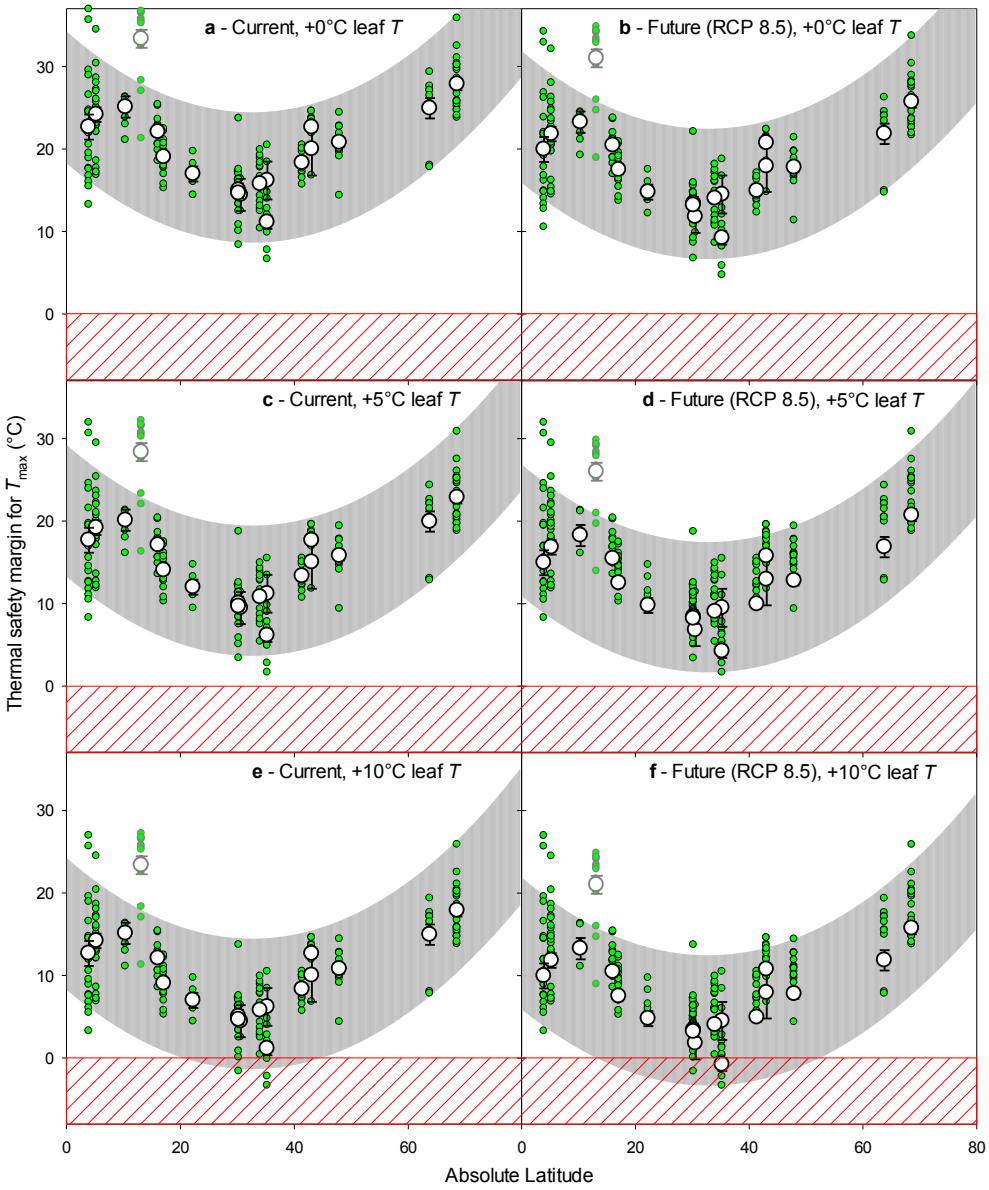

Table S1 Maximum increase in leaf temperatures above ambient air temperatures (ΔT) as reported in literature and unpublished sources.

Reference	Biome	Species	ΔT
Gates et al. 1968	Desert (North America)	*Opuntia* sp.	10-16°C
Smith 1978	Desert (North America)	Multiple	20°C
Valladares & Pearcy 1997	Chaparral (North America)	*Heteromeles arbutifolia*	10°C
Trubuzi 2005	Tropical (South America)	Multiple tropical species	>10°C
Doughty & Goulden 2008	Tropical (South America)	*Distachya huber*	5-10°C (forest canopy: 2.5-3°C)
Ishida et al.1999	Tropical (Southeast Asia)	*Macaranga conifera*	7°C
Vogel 2009	Multiple	multiple	5-20°C
Atkin (unpublished data)	Tropical rainforest (Australia)	*Cardwellia sublimis*	4.9°C
		Castanospermum australe	11.4°C
		Eleocarpus grandis	5.5°C
Miller 1972	Mangrove forest (North America)	*Rhizophora mangle*	12°C
Tyree & Wilmot 1990	Temperate forest (North America)	*Acer saccharum*	5-15°C
Leuzinger & Körner 2007	Temperate forest (Northern Europe)	Multiple	1-5°C (open canopy to closed canopy)
Leuzinger *et al*. 2010	Temperate urban (Northern Europe)	Multiple	4-10°C
Singsaas & Sharkey 1998	Temperate woodland (North America)	*Quercus alba*	8-10°C
Beadle *et al*. 1973	Agricultural (Northern Europe)	Corn and Sorghum	5-8°C
Ansari & Loomis 1959	Agricultural (North America)	Multiple	6-10°C (thin leaves) 20°C (thick leaves)

Table S1 references

Ansari A, Loomis W (1959) Leaf temperatures. *American Journal of Botany,* **46**, 713-717.

Beadle C, Stevenson K, Thurtell G (1973) Leaf temperature measurement and control in a gas-exchange cuvette. *Canadian Journal of Plant Science,* **53**, 407-412.

Doughty CE, Goulden ML (2008) Are tropical forests near a high temperature threshold? *Journal of Geophysical Research: Biogeosciences,* **113**, G00B07.

Gates DM, Alderfer R, Taylor E (1968) Leaf temperatures of desert plants. *Science,* **159**, 994-&.

Ishida A, Toma T, Marjenah (1999) Limitation of leaf carbon gain by stomatal and photochemical processes in the top canopy of *Macaranga conifera*, a tropical pioneer tree. *Tree Physiology,* **19**, 467-473.

Leuzinger S, Körner C (2007) Tree species diversity affects canopy leaf temperatures in a mature temperate forest. *Agricultural and Forest Meteorology,* **146**, 29-37.

Leuzinger S, Vogt R, Körner C (2010) Tree surface temperature in an urban environment. *Agricultural and Forest Meteorology,* **150**, 56-62.

Miller PC (1972) Bioclimate, leaf temperature, and primary production in red mangrove canopies in south Florida. *Ecology,* **53**, 22-45.

Singsaas EL, Sharkey TD (1998) The regulation of isoprene emission responses to rapid leaf temperature fluctuations. *Plant, Cell & Environment,* **21**, 1181-1188.

Smith WK (1978) Temperatures of desert plants: another perspective on the adaptability of leaf size. *Science,* **201**, 614-616.

Trubuzi ES (2005) Variações da temperatura foliar do dossel e o seu efeito na taxa assimilatória de CO_2 na Amazônia Central. PhD, Universidade de São Paulo, São Paulo, 84 pp.

Tyree MT, Wilmot TR (1990) Errors in the calculation of evaporation and leaf conductance in steady-state porometry: the importance of accurate measurement of leaf temperature. *Canadian Journal of Forest Research,* **20**, 1031-1035.

Valladares F, Pearcy RW (1997) Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll Heteromeles arbutifolia. *Plant Cell and Environment,* **20**, 25-36.

Vogel S (2009) Leaves in the lowest and highest winds: temperature, force and shape. *New Phytologist,* **183**, 13-26.

Table S2 Details of nearest meteorological stations to each of the sites where high temperature tolerance of leaf metabolism was measured. Negative altitude numbers indicate that the meteorological station was lower in altitude when compared to the field site. Also shown are various temperature metrics determined from nearby meteorological stations for each site. Only data from 2001-2010 was used, with the exception of data from the Andes site where data is only available from 2005 onwards.

Site	Distance to nearest met station km	Difference in altitude of nearest met station m	95th centile of daily max *T* (warmest month) °C	97.5th centile of daily max *T* (warmest month) °C	Mean max *T* of hottest 3 day period °C	Mean max *T* of hottest 5 day period °C
Toolik, Alaska	0	0	24.1	25.7	25.2	23.6
Umea, Sweden	3	-22	26.3	27.3	29.0	28.0
HWRC, Minnesota	19	98	32.6	34.1	34.1	33.1
Warra, TAS – low altitude	17	-33	30.4	32.1	35.0	31.4
Warra, TAS – high altitude	37	44	31.5	33.2	34.6	31.9
Black Rock Forest, New York	42	45	33.8	34.9	37.2	36.2
Acton, Canberra, ACT	9	6	37.8	39.3	39.9	38.2
Aranda, Canberra, ACT	11	-2	37.8	39.3	39.9	38.2
Calperum, SA	18	-15	43.0	44.8	44.8	44.2
Texas A&M, Texas	4	11	39.2	40.0	40.8	40.0
Great Western Woodlands, WA	93	-94	43.5	45.3	43.9	43.5
Jurien Bay, WA	11	-7	40.2	42.3	43.3	41.5
Alice Mulga, NT	44	16	42.7	43.9	43.0	42.0
Atherton, QLD	22	-112	35.7	36.6	37.0	36.1
Cape Tribulation, QLD	33	-21	39.6	41.3	37.3	36.8
Andes, Peru	0	0	21.5	22.4	20.8	20.4
Costa Rica	63	441	32.9	33.4	33.4	33.2
Paracou, French Guiana	78	-12	33.8	34.0	34.5	33.9
Iquitos, Peru	12	-11	36.8	37.4	37.5	37.0

Table S3 Percentage of species measured for which thermal safety margin of photosynthesis (T_{crit}) was exceeded under different climate scenarios and different elevations above ambient of leaf *T*s. Note that the columns titled "Current" and "RCP8.5" are identical to the columns in Table 2 and are included for easy comparison.

	Scenario														
Site	Current			RCP2.6			RCP4.5			RCP6.0			RCP8.5		
	Elevation in T_{leaf}			*Elevation in* T_{leaf}			*Elevation in* T_{leaf}			*Elevation in* T_{leaf}			*Elevation in* T_{leaf}		
	+0	+5	+10	+0	+5	+10	+0	+5	+10	+0	+5	+10	+0	+5	+10
Site															
Toolik, AK	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.0	15.0
Umea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HMRC, MS	0.0	0.0	20.0	0.0	0.0	66.7	0.0	0.0	66.7	0.0	0.0	60.0	0.0	6.7	86.7
TAS low alt	0.0	0.0	50.0	0.0	0.0	50.0	0.0	0.0	50.0	0.0	0.0	50.0	0.0	0.0	50.0
TAS high alt	0.0	10.0	60.0	0.0	10.0	90.0	0.0	30.0	100.0	0.0	30.0	90.0	0.0	30.0	100.0
BRF, NY	0.0	0.0	55.6	0.0	0.0	88.9	0.0	0.0	100.0	0.0	0.0	88.9	0.0	11.1	100.0
Aranda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cal. SA	5.6	66.7	100.0	5.6	77.8	100.0	5.6	88.9	100.0	5.6	88.9	100.0	5.6	88.9	100.0
Texas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GWW, WA	6.3	18.8	87.5	6.3	31.3	100.0	6.3	62.5	100.0	6.3	62.5	100.0	6.3	62.5	100.0
Jurien, WA	54.5	100.0	100.0	72.7	100.0	100.0	72.7	100.0	100.0	72.7	100.0	100.0	72.7	100.0	100.0
Alice, NT	0.0	40.0	80.0	0.0	40.0	80.0	0.0	40.0	80.0	0.0	40.0	80.0	0.0	40.0	100.0
Ath., QLD	0.0	7.1	42.9	7.1	14.3	78.6	7.1	14.3	78.6	7.1	14.3	78.6	7.1	14.3	85.7
Cape Trib.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Andes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. Rica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paracou	0.0	0.0	4.8	0.0	4.8	14.3	0.0	4.8	14.3	0.0	4.8	14.3	0.0	4.8	14.3
Iquitos	0.0	7.7	38.5	7.7	7.7	46.2	7.7	7.7	61.5	7.7	7.7	61.5	7.7	15.4	61.5
All sites	4.5	17.5	42.9	6.8	20.9	55.9	6.8	26.0	58.2	6.8	26.0	56.5	6.8	27.7	61.6

Table S4 Percentage of species measured for which thermal safety margin of respiration (T_{max}) was exceeded under different climate scenarios (RCP 2.6, 4.5, 6.0 and 8.5) and different elevations above ambient of leaf *T*s.

	Scenario														
Site	Current			RCP2.6			RCP4.5			RCP6.0			RCP8.5		
	Elevation in T_{leaf}			*Elevation in* T_{leaf}			*Elevation in* T_{leaf}			*Elevation in* T_{leaf}			*Elevation in* T_{leaf}		
	+0	+5	+10	+0	+5	+10	+0	+5	+10	+0	+5	+10	+0	+5	+10
Toolik, AK	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Umea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HWRC, MS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TAS low alt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TAS high alt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BRF, NY	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aranda	0.0	0.0	25.0	0.0	0.0	25.0	0.0	0.0	25.0	0.0	0.0	25.0	0.0	0.0	25.0
ANU	0.0	0.0	20.0	0.0	0.0	40.0	0.0	10.0	60.0	0.0	0.0	60.0	0.0	10.0	60.0
Cal. SA	0.0	0.0	0.0	0.0	0.0	5.6	0.0	0.0	5.6	0.0	0.0	5.6	0.0	0.0	5.6
Texas	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0
GWW, WA	0.0	0.0	6.3	0.0	0.0	12.5	0.0	0.0	12.5	0.0	0.0	12.5	0.0	0.0	12.5
Jurien, WA	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	9.1	0.0	0.0	9.1	0.0	0.0	9.1
Alice, NT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ath., QLD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cape Trib.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Andes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. Rica	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Paracou	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Iquitos	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3
All sites	0.0	0.0	1.8	0.0	0.0	4.0	0.0	0.4	4.9	0.0	0.0	5.3	0.0	0.4	5.3

Table S5. Regression equations expressing T_{max} & T_{crit} as function of other leaf traits & site climate, using species-means data. To select the best fitting equation from a group of input independent variables, data were explored using *Backwards-Stepwise Regression* – this revealed that chosen parameters exhibited *variance inflation factors* (VIF) less than 2.0 (i.e. minimal multi-collinearity); it also identified best-fit parameters (using *F*-to-remove criterion). Thereafter, multiple regression analyses were conducted to estimate predictive equations for the chosen variables. All selected variables were significant ($P<0.001$). The *PRESS* statistic (predicted residual error sum of squares) provides a measure of how well each regression model predicts the observations, with smaller *PRESS* indicating better predictive capability. Relative contributions of location and climate variables to each regression can be gauged from their standardized partial regression coefficients (β_1-β_2, depending on model equation). Abbreviations: aridity index (AI) calculated as the ratio of mean annual precipitation to mean annual potential evapotranspiration (UNEP, 1997, Zomer *et al.*, 2008); mean maximum temperature of the warmest month (MTWM); specific leaf area (SLA; ratio of leaf area to leaf mass – $m^2\ kg^{-1}$); leaf nitrogen (N, mg g^{-1}); leaf phosphorus (P, mg g^{-1}). MTWM at each site were obtained using site information and the *WorldClim* data base (Hijmans *et al.*, 2005).

Dependent variable	Input: independent variables *(Backwards-Stepwise Regression)*	Output: selected equations *(Multiple Linear Regression)*	*Multiple linear regression parameters*				
			n	r^2	*PRESS* statistic	Standardized partial regression coefficients	
						β_1	β_1
T_{crit}	MTWM, aridity index (AI)	T_{crit} = 35.228 + (0.412 * MTWM)	177	0.343	2765	0.586	
	SLA, [N], [P]	T_{crit} = 50.198 - (3.421 * [P])	152	0.197	3003	-0.444	
	SLA, [N], [P], MTWM, AI	T_{crit} = 35.228 + (0.412 * MTWM)	177	0.343	2765	0.586	
T_{max}	MTWM, aridity index (AI)	T_{max} = 48.175 + (0.308 * MTWM)	224	0.208	3408	0.456	
	SLA, [N], [P]	T_{max} = 59.770 - (2.865 * [P])	176	0.219	2339	-0.468	
	SLA, [N], [P], MTWM, AI	T_{max} = 52.698 - (1.551 * [P]) + (0.209 * MTWM)	176	0.288	2159	-0.253	0.339

Table S6 Seasonal changes in in high *T* tolerance [T_{max} and T_{crit}] and mean daily T of the 30 day period prior to measurements at two sites in Australia: Greater Western Woodland (GWW) in Western Australia, and Atherton in Far North Queensland, Australia. See Table 1 for details of each site. Values shown are means of 3-5 replicate plants. At each site, the same individual trees of each species were measured in both seasons, enabling insights to be made on the plasticity of each trait. Also shown is the extent to which T_{max} and T_{crit} changed per °C change in daily mean air *T* of the 30-days prior to measurement at each site (Δ per °C in daily *T*). For GWW, cool and warm season measurements took place in August (late winter) and March (late summer), respectively. For Atherton, cool and warm season measurements took place in Sept (late dry season) and April (late wet season), respectively.

Biome	Site	Species	Mean air *T* (30 days prior to measurement) (°C)		T_{max}			T_{crit}		
			Cool season	Warm season	Cool season	Warm season	Δ per °C in daily *T*	Cool season	Warm season	Δ per °C in daily *T*
Temperate woodland	GWW	*Olearia muelleri*	15.0	22.6	56.2 ± 1.2	58.0 ± 3.0	0.23	45.7 ± 0.1	50.0 ± 0.1	0.56
		Ptilotus holosericeus	14.8	22.6	58.2 ± 0.4	59.9 ± 1.4	0.21	49.5 ± 0.5	49.6 ± 1.3	0.02
		Sclerolaena diacantha	14.7	23.3	54.7 ± 1.2	59.9 ± 2.1	0.60	46.5 ± 1.4	49.30	0.33
Tropical rainforest	Atherton	*Alstonia muelleriana*	16.4	23.6	59.5 ± 0.6	61.2 ± 0.6	0.24	44.7 ± 0.7	50.9 ± 0.6	0.85
		Cryptocarya mackinnoniana	17.3	23.6	58.1 ± 0.9	58.8 ± 0.9	0.11	46.0 ± 0.4	48.6 ± 0.3	0.41
		Gillbeea adenopetala	17.1	23.6	54.0 ± 0.7	55.2 ± 0.3	0.18	47.20	48.5 ± 0.4	0.20